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Haemostatic studies in subarachnoid haemorrhage

MD Thesis

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Abstract

Object. The primary objective of this thesis was to establish the pattern of change in haemostatic systems in patients following a subarachnoid haemorrhage (SAH). I hypothesise that following a SAH there is an undefined period of increasing hypercoagulability, which if present would predispose to ischaemic stroke.

Methods. This was a prospective, observational study on 67 consecutive patients admitted with a primary diagnosis of SAH. There were 24 males, median age 47.5 years (25-75) and 43 females, median age 53 years (23-80).

Blood was taken at 4 time periods (<48hours, 4-5, 9-10 and 15-16 days) following the ictus depending on the day of hospital admission, and on regular intervals during the hospital stay. In addition, a sample was taken at 3 months from the ictus. A Thromboelastograph (TEG) profile performed at 37°C, and the routine coagulation studies, International Normalised Ratio (INR) and Activated Partial Thromboplastin Time Ratio (APTR) were obtained at each of these time points. In addition a full blood count, biochemical profile, and plasma for coagulation and fibrinolytic assays was also taken.

Results. The results demonstrated that SAH patients were hypercoagulable immediately following the ictus, when compared with the blood sample taken 3 months later. In addition we observed the development of an increasingly hypercoagulable state for the first 21 days following the ictus. This increase in coagulation was demonstrated against a background of haemodilution during this time.

Conclusions. This highly significant data demonstrates that SAH patients become increasingly hypercoagulable over time (maximum 21 days) following the ictus. This prothrombotic tendency has reversed by 3 months. This may provide a new direction in the treatment of symptomatic vasospasm.

In addition, an in-vitro study using TEG has been performed in 20 volunteer subjects to assess whether haemodilution 'per se' has an intrinsic affect on coagulation specific to the diluent itself. This study demonstrates that haemodilution does alter coagulation profiles measured using TEG. Different crystalloid and colloid fluids used to achieve haemodilution produce qualitatively consistent but quantitatively very different effects on coagulation in-vitro.

Dedication

To Sue, Bill, Peter, Jane, Sarah, Helen and David.

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Abbreviations list

ACA – Anterior cerebral artery

ADP – Adenosine diphosphate

ANOVA – Analysis of variance

APC – Activated protein C

APTT – Activated partial thromboplastin time

AVM – Arteriovenous malformation

AVP – Arginine vasopressin

C – Complement

CaCl₂ – Calcium chloride

CBF – Cerebral blood flow

CI – Confidence interval

CSF – Cerebrospinal fluid

CT – Computerised tomography

CVP – Central venous pressure

CWB – Citrated whole blood

Dex - Dextran

DIC - Disseminated intravascular coagulation

DIND – Delayed ischaemic neurological deficit

DVT – Deep vein thrombosis

ET – Endothelin

FBC – Full blood count

FDP – Fibrin degradation products

G – Sheer elastic modulus (dynes/cm²)

GCS – Glasgow coma score

GEL – Gelfusion/Gelofusine

GOS – Glasgow outcome score

GP - Glycoprotein

HA – Human albumin

Hb – Haemoglobin

Hct – Haematocrit

HES – Hydroxyethyl starch

HHH - Hypertension/hypervolaemia/haemodilution

HRT – Hormone replacement therapy

ICP – Intracranial pressure

ICS – Intracellular space

Ig – Immunoglobulin

IL – Interleukin

INR International normalised ratio

ISS – Interstitial space

IVS – Intravascular space

LY – Lysis time

MA – Maximum amplitude

MCA – Middle cerebral artery

NS – Normal saline

OCP – Oral contraceptive pill

PAF – Platelet activating factor

PAI – Plasminogen activator inhibitor

PAP – Plasmin-Antiplasmin

PCV – Packed cell volume

PE – Pulmonary embolus

PF – Prothrombin fragment

PG – Prostaglandin

Plt - Platelet

PMN – Polymorphonuclear neutrophils

PNAH – Peroperative normovolaemic acute haemodilution

PPF – Plasma protein fraction

PS – Protein S

PT – Prothrombin time

RCC – Red cell count

SAH – Subarachnoid haemorrhage

SD – Standard deviation

TAT – Thrombin-Antithrombin complex

TCD – Transcranial doppler

TF – Tissue factor

TFPI – Tissue factor pathway inhibitor

TIA – Transient ischaemic attack

TNF – Tumour necrosis factor

TPA – Tissue plasminogen activator

UPA – Urokinase-like plasminogen activator

VWF – Von Willebrand Factor

WCC – White cell count

WFNS – World Federation of Neurosurgeons

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Introduction

This thesis commences with a discussion of modern theories of blood coagulation in man and its control in vivo. I then go on to describe the standard, routine coagulation tests performed clinically to assess coagulation mechanisms, and their limitations.

I then describe in detail the principles of thromboelastography (TEG), a coagulation test suggested to be the most sensitive assessment of hypercoagulation. I explain the significance of each of the TEG variables, how they are affected by particular blood components, and how the test is complimentary to the data generated in standard clinical tests of coagulation.

I then discuss how TEG has been used previously in experimental and clinical studies.

TEG forms the cornerstone of the experimental studies into coagulation changes following subarachnoid haemorrhage (SAH) in this thesis. Thus, the thesis commences with 3 control studies into this technique. The first assesses the reproducibility of a TEG measurement from a single citrated blood sample in a control population. The second aims to ascertain the range of TEG variables in a control population, and examines whether age and/or sex may affect its value. There is then a discussion of the evidence for a sex difference in coagulation mechanisms, based on the results of the study. Finally, the reproducibility of a TEG measurement in a single subject over time is assessed in a control population. This was central to the clinical studies where serial measurements of coagulation were obtained over time following a SAH. This study is followed by a short discussion of the evidence for cyclical or circadian change in coagulation in man.

This section is followed by the experimental studies into haemostasis following subarachnoid haemorrhage, which form the central part of the thesis. This commences with a comprehensive literature review of previous work in this and related fields.

There is first an explanation of subarachnoid haemorrhage (SAH), its incidence and aetiology. Then an explanation of how coagulation mechanisms and changes may have a direct impact on the outcome of patients with SAH and a review of previous haematological studies in the SAH.

There is then a discussion of the previous work in related neurosurgical fields. This deals with coagulation changes following intracerebral haemorrhage and head injury, and explains potential mechanisms by which intracranial pathology may affect coagulation mechanisms. This is followed by the more widely studied field of ischaemic stroke and how coagulation manipulation is used as a primary form of management in this condition.

There is then a review of one of the major controversies in SAH, the timing of aneurysm surgery. This is directly relevant to the present studies. If coagulation could be manipulated so that patients are hypercoagulable to the point at which an aneurysm is secured following a SAH, but then anticoagulated during the period of maximal ischaemia, this may have a direct impact on patient outcome. Previous work to prevent rebleeding from intracranial aneurysms using antifibrinolytic drugs is presented.

The greatest cause of morbidity following SAH is cerebral ischaemia. In the presence of cerebral ischaemia, hypercoagulation may predispose to a worse neurological outcome, through further ischaemic deficit. The

mechanisms, management and outcome from cerebral ischaemia following SAH are discussed comprehensively.

Recently a novel new method of securing cerebral aneurysms following SAH has become available as an alternative to intracranial surgery and aneurysm clipping. Endovascular coiling is becoming increasingly utilised in neurosurgical centres. Thus, there is then a discussion of how surgery as opposed to endovascular therapy may affect coagulation and cerebral ischaemia following SAH.

There is then a review of the more general topic of the metabolic response of the human body to trauma. SAH and surgery provide a major trauma to the human body, and homeostatic mechanisms may be directly relevant to results of these experimental studies.

The discussion is then broadened to include previous work in the field of coagulation and surgery in general. Followed by previous studies into coagulation changes and other neurosurgical procedures, excluding aneurysm surgery following SAH. This includes a review of previous work in the surgical field using TEG.

The literature review concludes with sections dealing with how issues related to the management of SAH patients may directly affect coagulation mechanisms. This includes the affects of anaesthetic agents, intravenous fluids, gender and immobilisation, together with other risk factors for thrombosis in the surgical patient.

Experimental study 1 examines how the standard haematological indices (haemoglobin concentration, haematocrit, red cell count, white cell count and platelet count), and the standard coagulation indices (prothrombin time and

activated partial thromboplastin time) change serially following a SAH in a consecutive series of 67 patients.

Experimental study 2 examines how the TEG variables R, K, Angle, MA, TMA and G change serially following a SAH.

Experimental study 3 examines how the plasma markers of coagulation (thrombin-antithrombin and prothrombin fragments 1+2) and fibrinolysis (plasmin-antiplasmin and d-dimer), and source fibrinogen change serially following a SAH.

Experimental study 4 examines whether intracranial surgery performed during the period following a SAH affects the coagulation profile in these patients.

It has been suggested that the severity of neurological deficits resulting from cerebral ischaemia following a SAH correlates with the amount of basal subarachnoid blood on the admission CT scan. Experimental study 5 examines whether haemostatic parameters (measured using TEG) reflect the severity of subarachnoid blood on the admission CT (measured using Fisher grading). In addition the serial TEG data is examined for the confounding variables of age and gender.

There then follows a discussion of the findings of experimental studies 1 to 5 and conclusions.

The final part of this thesis looks at a central part in the management of SAH. So-called 'triple H' therapy (Hypertension, hypervolaemia and haemodilution) has become the definitive management strategy in preventing ischaemic outcome following SAH. The core of this theory is intravascular volume loading, but controversy exists as to which fluid regimens best achieve this,

and experimental evidence has proved contradictory. Experimental study 6 examines in-vitro using TEG whether different crystalloid and colloid infusion fluids have an intrinsic effect on coagulation, specific to the diluent itself, and thus may be contributing to the observed changes in coagulation in patients following SAH.

Haemostasis

'Normal haemostasis - the controlled activation of clot formation and clot lysis that stops haemorrhage without permitting inappropriate thrombosis'

Haemostasis is a physiological mechanism that maintains blood in a fluid state within the circulation and the process whereby haemorrhage following vascular injury is arrested (Fig. 1). The coagulation of blood is mediated by cellular components and soluble plasma proteins. In response to vascular injury, circulating platelets adhere, aggregate, and provide cell surface phospholipid for the assembly of blood clotting enzyme complexes (Fig. 2). The extrinsic pathway of blood coagulation is initiated when blood is exposed to non-vascular cell bound tissue factor in the subendothelial space. Tissue factor binds to activated factor VII, and the resulting enzyme complex activates factors IX and X of the intrinsic and common coagulation pathways respectively. Factor IX activated by the tissue factor pathway in turn activates additional factor X, in a reaction which is greatly accelerated by a cofactor, factor VIII. Once activated, factor X converts prothrombin to thrombin (factor IIa) in a reaction that is accelerated by factor V. In the final step of the coagulation pathway, thrombin cleaves fibrinogen to generate fibrin monomers, which then polymerise and link to one another to form a chemically stable clot. Thrombin also feeds back to activate cofactors VIII and V, thereby amplifying the coagulation mechanism.

The blood coagulation cascade has the ability to transduce a small initiating stimulus into a large fibrin clot. The potentially explosive nature of this cascade is offset by natural anticoagulant mechanisms. The maintenance of

adequate blood flow and the regulation of cell surface activity limit the local accumulation of activated blood clotting enzymes and complexes. Antithrombin III is a plasma protein that inhibits the activity of the serine proteases of the intrinsic and common coagulation pathways. In the presence of endogenous heparan sulphate, the rate of inactivation is increased by a factor of several thousand. In the presence of thrombomodulin bound to endothelial cells, thrombin activates protein C, which in turn cleaves activated factors VIII and V. Like other reactions in haemostasis, this one is accelerated by a cofactor, in this case protein S. The tissue factor pathway inhibitor is a lipoprotein associated plasma protein that forms a quaternary complex with tissue factor and activated factors VII and X, thereby inhibiting the extrinsic coagulation pathway. Finally, a series of linked enzymatic reactions generates plasmin, a serine protease that acts on fibrin to dissolve preformed clots.

Congenital and acquired hypercoagulable states arise when there is an imbalance between the anticoagulant and prothrombotic activities of plasma in which the prothrombotic activities predominate (Rosing et al., 2001). The mechanisms that underlie the thrombotic phenotype are defined by Virchow's triad (Virchow, 1856), these are; a decrease in blood flow, injury to the vessel wall, and a change in the systemic balance of procoagulant and anticoagulant factors. One might therefore predict that the loss of a certain anticoagulant would cause a shift in the haemostatic balance and thereby promote a diffuse thrombotic diathesis. This prediction however, does not hold true. In fact, systemic alterations in the haemostatic mechanism typically give rise to local thrombotic lesions in discrete segments of the vascular tree.

The pathophysiological basis for these observations is poorly understood. The conventional wisdom is that the focal lesions are attributable to superimposed defects in the vascular wall or blood flow. In other words, the phenotypic state of systemic hypercoagulable states rests on the ability of these two local mechanisms to compensate for a uniform change in the haemostatic balance. It is now believed that the focal nature of thrombotic lesions is better understood in the context of signalling pathways specific to the vascular bed.

According to this model, the endothelium integrates different extracellular signals and cellular responses in different regions of the vascular tree. On the one hand, the endothelium is exposed to diverse environmental cues, including those from integrins and growth factors, haemodynamic forces, and cell to cell signalling. On the other hand, the ability of the endothelium to transduce a given signal is regulated in both space and time. As a result of these variables, endothelial cell derived procoagulant and anticoagulant activities are differentially expressed throughout the vascular tree. The focal thrombotic phenotype that is associated with a systemic loss of anticoagulant function reflects the critical role of that particular mechanism in restricted sites of the vasculature.

Fibrinolysis (Fig. 3) is the process whereby fibrin is degraded by plasmin. The circulating proenzyme plasminogen is activated to plasmin following injury by tissue plasminogen activator (TPA) and urokinase-like plasminogen activator (UPA) released from damaged or activated cells.

Plasmin digests fibrin (or fibrinogen) into fibrin degradation products (FDPs) and also degrades factors V and VII. Free plasmin is inactivated by plasma α_2 -antiplasmin and α_2 -macroglobulin.

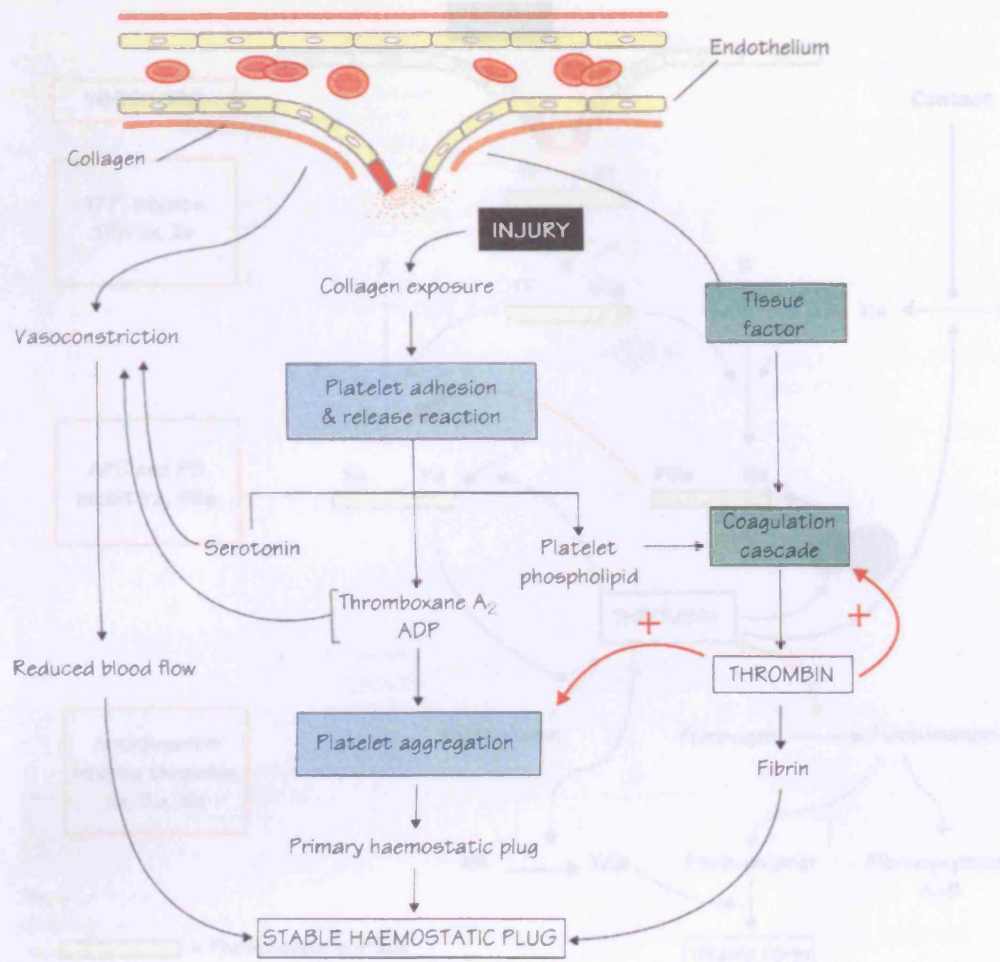


Figure 1. The haemostatic response to injury.

ADP – Adenosine diphosphate

Taken from: Mehta, A and Hoffbrand V. Haematology at a Glance. 2000. Blackwell, Oxford, UK.

Factor VII. Extrinsic pathway, Factor VII. Intrinsic pathway, Factors XI, IX, VIII. Common pathway, Factors X, V, II, and fibrinogen.

VWF – von Willebrand factor, TFFI – Tissue factor pathway inhibitor, APC – Activated protein C, PS – Protein S.

Taken from: Mehta, A and Hoffbrand V. Haematology at a Glance. 2000. Blackwell, Oxford, UK.

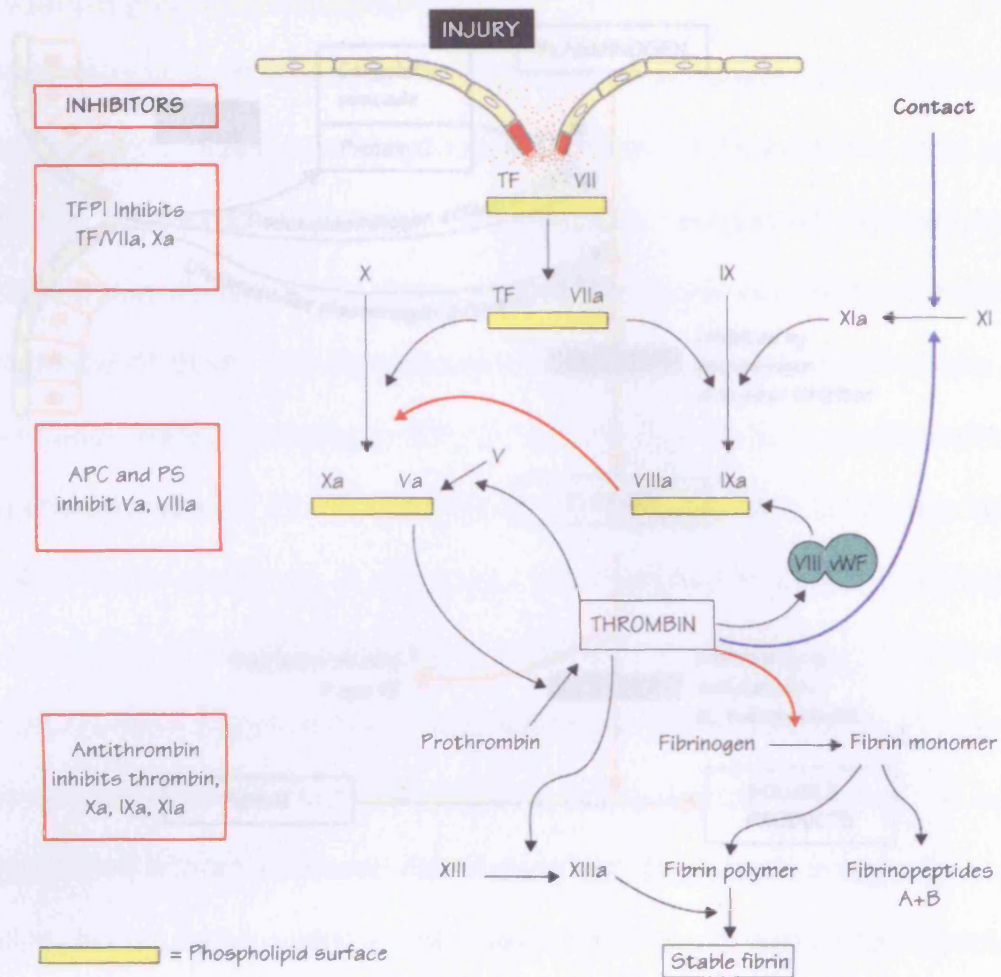


Figure 3. Fibrinolysis.

Injury causes release of TPA and UPA which, together with activated components from the coagulation pathway and protein C, activate plasminogen to plasmin.

Figure 2. The coagulation pathway.

Injury initiates release of tissue factor (TF). Tissue factor binds and activates Factor VII. Extrinsic pathway, Factor VII. Intrinsic pathway, Factors XI, IX, VIII. Common pathway, Factors X, V, II, and fibrinogen.

VWF – von Willebrand factor, TFPI – Tissue factor pathway inhibitor, APC – Activated protein C, PS – Protein S.

Taken from: Mehta, A and Hoffbrand V. Haematology at a Glance. 2000. Blackwell, Oxford, UK.

Hypercoagulability in humans

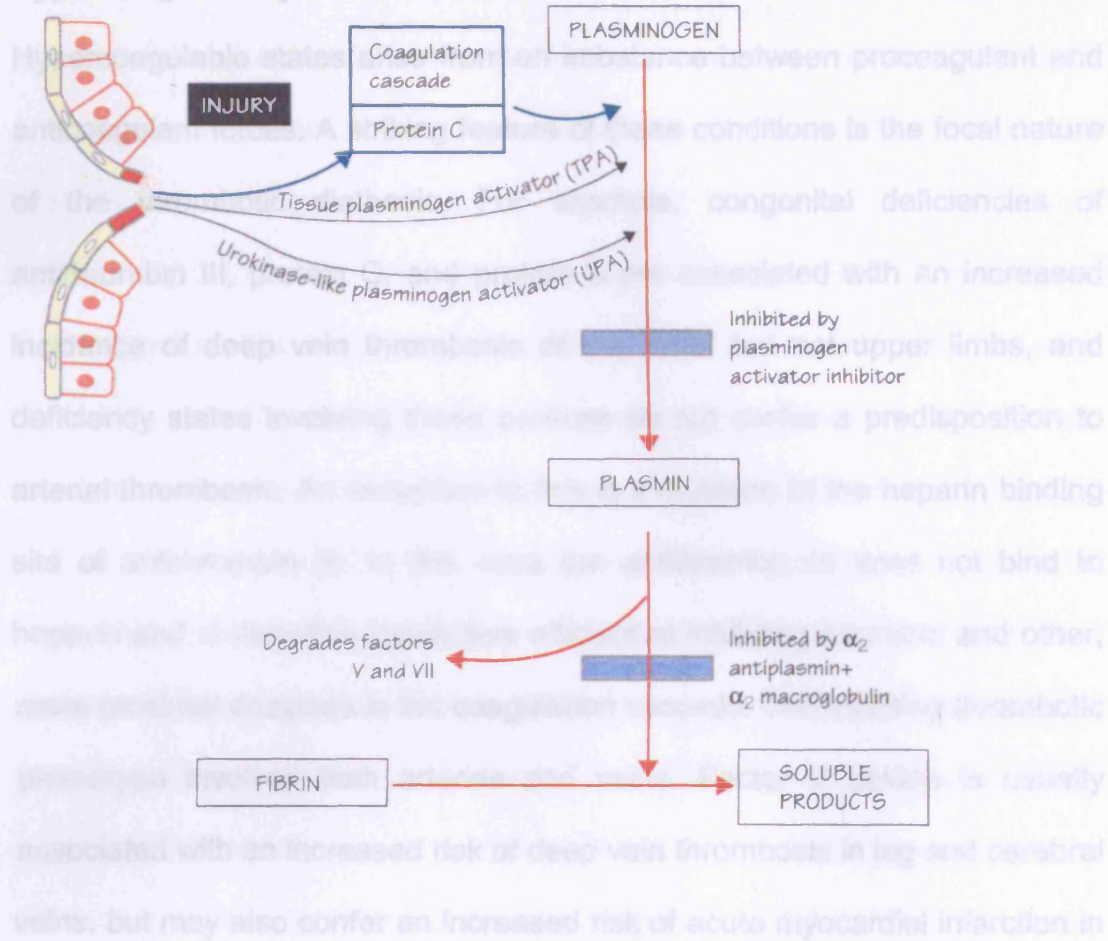


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Several acquired hypercoagulable states are also associated with vascular bed specific thromboses. Paroxysmal nocturnal haemoglobinuria and the myeloproliferative diseases are characterised by an unusually high incidence of thromboses of the hepatic, portal and mesenteric veins. Patients with antiphospholipid antibody syndrome have a propensity to form clots within deep vein thromboses and arterial segments of the vascular tree, including blood vessels of the retina and placenta. Warfarin induced necrosis of the skin is associated with extensive thrombosis of the postcapillary venules and small

Hypercoagulability in humans

Hypercoagulable states arise from an imbalance between procoagulant and anticoagulant forces. A striking feature of these conditions is the focal nature of the thrombotic diathesis. For example, congenital deficiencies of antithrombin III, protein C, and protein S are associated with an increased incidence of deep vein thrombosis of the lower but not upper limbs, and deficiency states involving these proteins do not confer a predisposition to arterial thrombosis. An exception to this is a mutation of the heparin binding site of antithrombin III. In this case the antithrombin III does not bind to heparin and is therefore much less efficient at inhibiting thrombin and other, more proximal enzymes in the coagulation cascade. The resulting thrombotic phenotype involves both arteries and veins. Factor V Leiden is usually associated with an increased risk of deep vein thrombosis in leg and cerebral veins, but may also confer an increased risk of acute myocardial infarction in young women who smoke. The prothrombin G20210A mutation predisposes patients to deep vein thrombosis in the legs and brain, and may be a genetic risk factor for both stroke and ischaemic heart disease.

Several acquired hypercoagulable states are also associated with vascular bed specific thrombosis. Paroxysmal nocturnal haemoglobinuria and the myeloproliferative diseases are characterised by an unusually high incidence of thrombosis of the hepatic, portal and mesenteric veins. Patients with antiphospholipid antibody syndrome have a propensity to form clots within particular venous and arterial segments of the vascular tree, including blood vessels of the retina and placenta. Warfarin induced necrosis of the skin is associated with extensive thrombosis of the postcapillary venules and small

veins within subcutaneous adipose tissue. The microthrombotic lesions characteristic of thrombotic thrombocytopenic purpura and the haemolytic uraemic syndrome are detectable in all organs with the notable exception of the liver and the lungs.

On the one hand, each of these disorders is characterised by a systemic dysfunction of the haemostatic pathway resulting in the absence of a circulating natural anticoagulant or the presence of an activated cell surface membrane. On the other hand, each condition is associated with a unique and restricted phenotype. How can these diverse responses to a systemic problem be explained? According to Virchow's triad, the phenotype must arise from local changes in blood flow, disruption of the vascular wall, or vascular bed restricted alterations in the balance of anticoagulant and procoagulant factors.

Blood flow limits the extent of the procoagulant response, and its relative deficiency may underlie the tendency for thrombi to develop in certain parts of the vascular tree. However, blood flow is not the principal determinant of haemostatic control in all vascular beds. Local disruption of the integrity of the blood vessel wall may also have an important effect on certain thrombotic phenotypes. For example, surgery puts patients with congenital deficiencies of protein C, protein S, or antithrombin III at particularly high risk of perioperative deep vein thrombosis. However, similar procedures in patients with factor V Leiden deficiency do not confer an increased risk of thrombosis. Moreover, the loss of integrity of the vessel wall caused by the rupture of a coronary atheromatous plaque does not lead to a higher rate of occlusive thrombus in patients with defects of protein C, protein S, or antithrombin III

pathways. Indeed the very absence of association between the loss of these natural anticoagulant mechanisms and the incidence of myocardial infarction suggests that these particular pathways are relatively unimportant contributors to the haemostatic balance of the coronary vasculature. Such epidemiological observations provide strong support for the existence of regionally distinct haemostatic balances.

In addition, a further explanation for the focal nature of haemostatic control is found in the uneven distribution of various anticoagulant and procoagulant factors throughout the vascular tree(Rosenberg, Aird, 1999).

The mechanisms favouring hypercoagulability may differ following different forms of trauma. In addition, it appears likely that post traumatic hypercoagulability is a systemic phenomenon(Dahl, 1999).

Thromboplastin. Although the brain contains the highest concentration of thromboplastin in the human body(Bjorklid et al., 1977; Bjorklid, Storm, 1977), and it is speculated that trauma, infarction and surgery to the brain results in the release of tissue thromboplastin, and activation of the coagulation cascade, there is no direct evidence to support this(Hamilton et al., 1994).

Tissue Factor. Tissue factor (TF) is the primary cellular initiator of blood coagulation and the most potent trigger of blood coagulation known today. The adventitia surrounding the major blood vessels, the brain, and the bone marrow are the structures with high levels of TF(Dahl, 1999). The concentration of TF is reported to be greater in the cortical grey matter than in white matter(del-Zoppo et al., 1992). It is reported to be associated with non capillary microvessels in the cerebral cortex and with the adventitia, as

seen in superficial cerebral vessels(Dahl, 1999; Fujii et al., 2001). Thus, the entry of blood into the subarachnoid space may cause a considerable amount of TF release into the systemic circulation through injury to superficial brain tissues, including superficial cerebral arteries, resulting in systemic haemostatic activation.

Endothelial cells. Endothelial cells lose their non-thrombogenic properties when stimulated by thrombin and certain cytokines, which trigger synthesis and expression of TF on their luminal and subcellular surfaces. Tissue factor pathway inhibitor, the major down regulator of the procoagulant activity of tissue factor-factor VIIa, is synthesised and secreted by the endothelial cells. The protein C anticoagulant pathway plays a critical role in controlling blood coagulation. The receptor for protein C is bound to the endothelium and accelerates protein C activation. It is known that this receptor can be down regulated by pro-inflammatory cytokines, such as IL-1 β .

Vascular bed specific signalling pathways

The endothelium is involved in a wide range of homeostatic processes, including the maintenance of blood fluidity, the control of vasomotor tone, and the transfer of nutrients and cells between blood and the underlying tissue. On the anticoagulant side the endothelium releases heparan sulphate and prostacyclin, expresses thrombomodulin, tissue type plasminogen activator, tissue factor pathway inhibitor, and endothelial nitric oxide synthase, and provides a non thrombogenic cell surface membrane. On the procoagulant side, the endothelial cells release von Willebrand factor and plasminogen activator inhibitor type 1, express receptors for cell surface tissue factor and thrombin, expose critical binding sites for coagulation factor

complexes, and attracts platelets and monocytes to sites of activation. Under normal conditions, a delicate balance between the anticoagulant and procoagulant activities of the endothelium is achieved by a series of regulatory linking mechanisms. These mechanisms are capable of integrating multiple signals to generate a response that varies both in space and time, so that within any given segment of the vascular tree, the endothelium is capable of shifting the haemostatic balance from moment to moment. The temporal and spatial nature of these regulatory mechanisms endows the haemostatic system with tremendous flexibility. At the same time, these very properties make the endothelium vulnerable to focal dysfunction and pathophysiological disorders.

Extracellular signals

There are an array of signals residing in the microenvironment that regulate the procoagulant and anticoagulant properties of the endothelium. These signals include growth factors, cytokines, mechanical forces, circulating lipoproteins, coagulation factors, components of the extracellular matrix, and neighbouring cells. The extracellular signals are transduced by endothelial cell signalling networks, resulting in alterations in procoagulant and anticoagulant mRNA, protein and function.

Cell subtype specific signalling pathways

A second mechanism that contributes to the generation of vascular bed specific phenotypes is signalling pathways specific to cell subtype. Endothelial cells from various vascular beds have different responses to the same signals. In addition, the signalling pathways for endothelial cells involve regulating a whole series of gene networks.

Transcription regulation

A final mechanism underlying the generation of vascular bed specific phenotypes is found at the level of transcription. Transcription control is a regulatory linking mechanism. It is generally believed that tissue specific genes are regulated by common mechanisms in different cells of the same lineage. The overall expression of a single gene may be mediated by distinct vascular bed specific signalling pathways that begin in the extracellular milieu and end in separate sites on the promoter gene region of the gene. This expanded repertoire of interactions between DNA and protein provides the endothelium with an even greater capacity for integrating multiple extracellular signals.

Principles of Thromboelastography

The use of Thromboelastography (TEG), an in-vitro bench test in monitoring haemostasis is based on two assumptions:

The end result of the haemostatic process is the production of a clot.

The clot's physical properties (rate, strength, and stability) can therefore be used to assess the haemostatic process.

TEG measures the clots physical properties by the use of a special cylindrical cup that holds the blood and is oscillated through an angle of 4° $45'$ (Fig. 4). Each rotation cycle lasts 10 seconds. A pin is suspended in the blood by a torsion wire that is monitored for motion. This wire acts as a strain gauge. The torque of the rotating cup is transmitted to the immersed pin only after fibrin-platelet bonding has linked the cup and the pin together. The strength of these fibrin-platelet bonds affects the magnitude of the pin motion. Thus, the magnitude of the output is directly related to the strength of the formed clot. As the clot retracts or lyses, these bonds are broken and the transfer of cup motion is diminished. The rotation of the pin is converted to an electrical signal which is monitored by computer(Mallett, Cox, 1992).

The whole haemostatic process from the time of the initial platelet-fibrin interaction, through platelet aggregation, clot strengthening and fibrin cross linkage right through to eventual clot lysis can be assessed from the TEG tracing (Fig. 5). The diagnosis of coagulation factor activity deficiency, platelet abnormalities, dysfibrinogenaemias, fibrinolysis, or diffuse intravascular coagulation can all be made from a single thromboelastogram(Tuman et al., 1987).

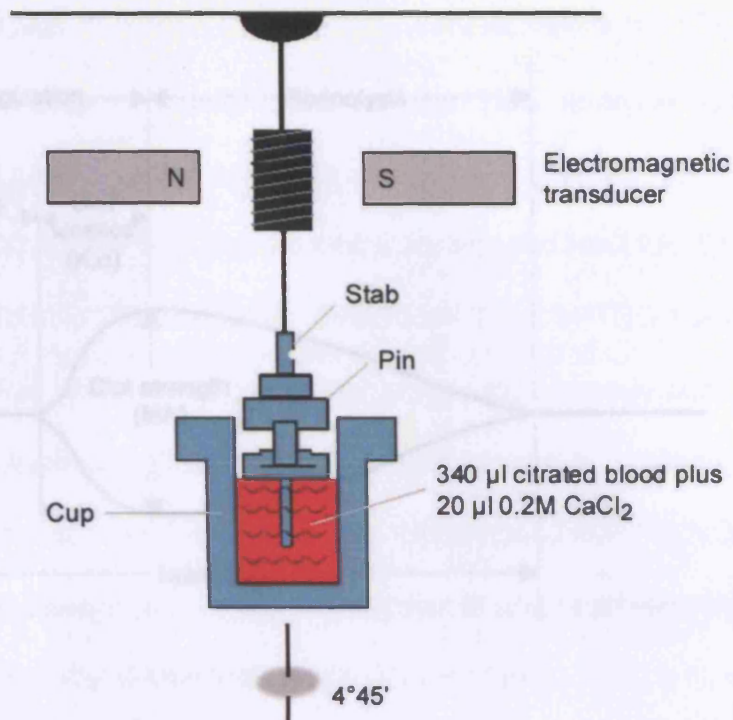


Figure 4. Schematic diagram of TEG apparatus.

The cup rotates about the pin every 10 secs. As the fibrin strands start to form they couple the motion of the pin to the cup. This sets up a strain on the wire which is read by an electromagnetic transducer to produce a characteristic TEG trace.

TEG parameters

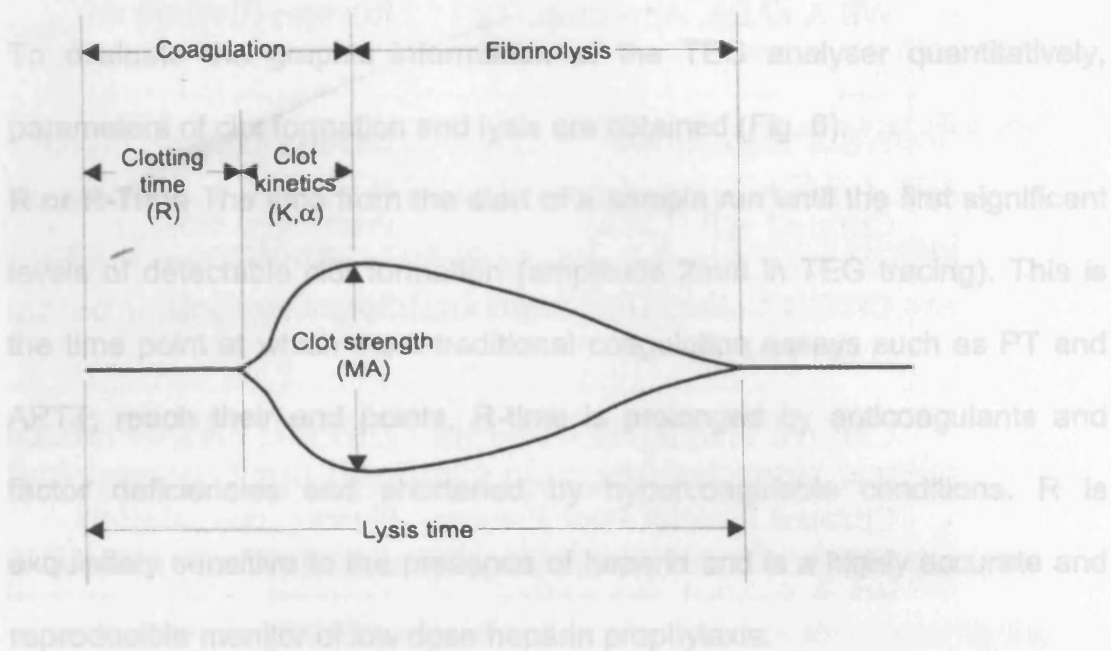


Figure 5. The TEG tracing.

This schematic representation of a TEG trace shows how TEG allows an assessment of the whole haemostatic process from the onset of coagulation to fibrinolysis.

R or R-Time The time from the measurement of R until a fixed level of clot strength is reached. R is highly sensitive to the presence of heparin and is a highly accurate and reproducible monitor of low dose heparin prophylaxis.

Angle (α) The angle is closely related to K-time, since they are both a function of the rate of polymerisation. The angle is more comprehensive than K-time, since there are hypocoagulable conditions in which the final level of clot firmness does not reach an amplitude of 20mm.

MA - Maximum Amplitude: Measurement of the maximum strength of the developed clot. Clot strength is the result of platelet number and function, and to a lesser extent by fibrin. Platelet abnormalities whether qualitative or quantitative substantially disturb the MA.

TMA: Time to maximum amplitude.

TEG parameters

To evaluate the graphic information of the TEG analyser quantitatively, parameters of clot formation and lysis are obtained (Fig. 6).

R or R-Time The time from the start of a sample run until the first significant levels of detectable clot formation (amplitude 2mm in TEG tracing). This is the time point at which most traditional coagulation assays such as PT and APTT, reach their end points. R-time is prolonged by anticoagulants and factor deficiencies and shortened by hypercoagulable conditions. R is exquisitely sensitive to the presence of heparin and is a highly accurate and reproducible monitor of low dose heparin prophylaxis.

K or K-Time The time from the measurement of R until a fixed level of clot firmness is reached (amplitude 20mm). K-time is a measure of the speed of clot kinetics to reach a certain clot strength. K is thus shortened by increased fibrinogen and to a lesser extent by platelet function, and is prolonged by anticoagulants that affect both.

Angle (α) The angle is closely related to K-time, since they are both a function of the rate of polymerisation. The angle is more comprehensive than K-time, since there are hypocoagulable conditions in which the final level of clot firmness does not reach an amplitude of 20mm.

MA Maximum Amplitude. Measurement of the maximum strength of the developed clot. Clot strength is the result of platelet number and function, and to a lesser extent by fibrin. Platelet abnormalities whether qualitative or quantitative substantially disturb the MA.

TMA Time to maximum amplitude.

G Shear elastic modulus strength measured in dyn/cm^2 . If A is the amplitude of the sample it is calculated by the formula $G=5000A/(100A)$. Note that A is equal to MA until MA is reached. The value of G increases exponentially in proportion to the amplitude (A) of the TEG tracing, and is more sensitive to small changes in clot strength or breakdown than is the amplitude in mm.

It is important to appreciate that an essential difference between the clotting of blood in vivo and the clotting of blood in the thromboelastogram is the presence/absence of the vessel wall. If the haemostatic defect is due to interference with the normal interaction of the platelets with the vascular endothelium, rather than with the platelet aggregation responses or platelet involvement in the coagulation sequence, then the TEG MA will not be affected(Mallett, Cox, 1992). It is for this reason that the effect of certain anti-inflammatory agents such as aspirin on platelets are not demonstrated by TEG(Gibbs, Sear, 1995).

The MA parameter of the TEG, and to a lesser extent the K time, has been shown by Oshita and colleagues(Oshita et al., 1999a) to be directly related to the platelet count in diluted plasma samples.

As well as providing data on the initiation of coagulation, if the trace is allowed to proceed it can also supply information detailing the process of fibrinolysis. The LY30 and LY60 parameters measure percent lysis at 30 and 60 minutes after MA is reached. The LY30 and LY60 measurements are based on a reduction of the area under the TEG tracing from the time MA is measured until 30 (or 60) minutes after MA. The A30 and A60 are point

measures of amplitude at time points after MA. The lysis time provides more information about the process of fibrinolysis as a whole.

Figure 7 demonstrates the value of lysis time. Thirty minutes after MA, the amplitudes of both tracings read zero. However, the LY30 parameters of the two tracings are radically different. In the top tracing, the shaded area under the curve is approximately 15% of the rectangular area (the rectangle represents the area under the curve if there had been no fibrinolysis). Thus, the LY30 is approximately 85%. In the bottom tracing, the shaded area comprises about 85% of the rectangle. This makes the LY30 approximately 15%.

The A30 and A60 represent the fibrinolytic status at exactly 30 and 60 minutes after MA is reached. LY30 and LY60 represent the fibrinolytic process that took place during those 30 or 60 minutes.

TEG can also be used to monitor the effects of antifibrinolytic therapy in the clinical setting (Mallett, Cox, 1992) and has become a routine haematological index in hepatic and cardiac surgery.

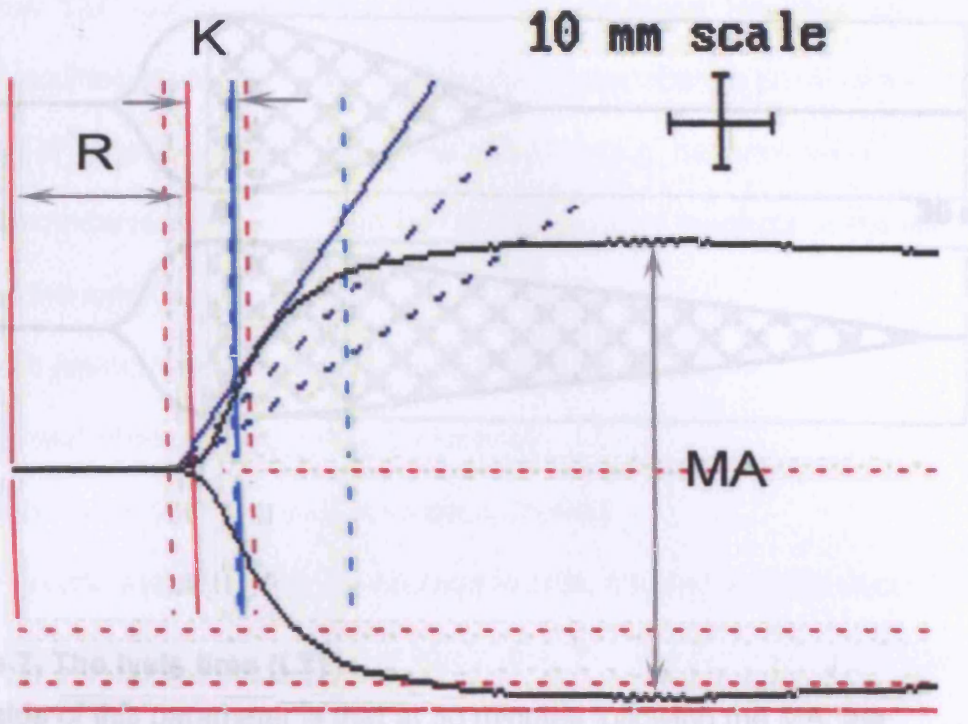


Figure 7. The top trace is the TEG tracing of a patient with normal coagulation. The bottom trace is the TEG tracing of a patient with disseminated intravascular coagulation (DIC). The amplitude of the 2 traces is zero. However, in the top trace far more fibrinolysis has taken place. This is only appreciated by calculating the lysis time.

Figure 6. The TEG tracing parameters of coagulation R, K, Angle and MA.

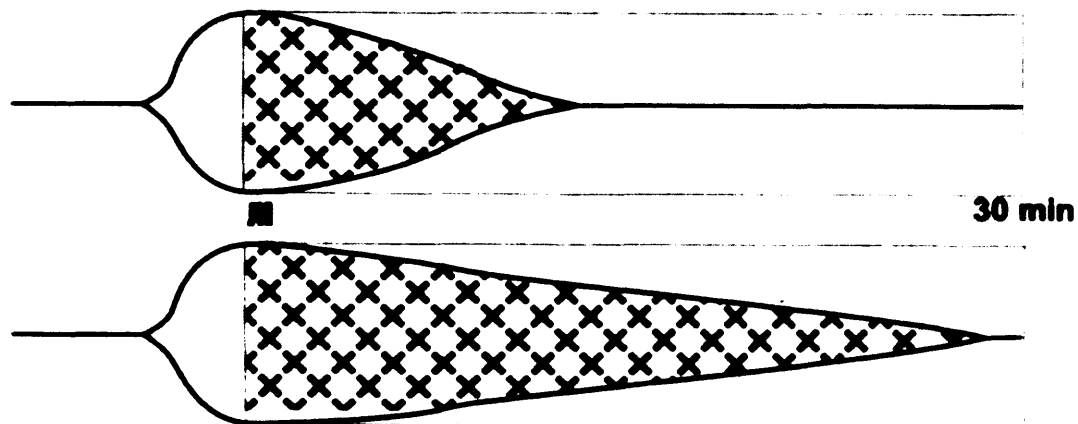


Figure 7. The lysis time (LY).

The value of this parameter is that at 30 minutes following the MA, the amplitude of the 2 traces is zero. However, in the top trace far more fibrinolysis has taken place. This is only appreciated by calculating the lysis time.

TEG sample types

In general TEG can be performed on native whole blood. However, samples can be modified *in vitro* to speed up analysis, determine if a possible therapy might be of benefit, or reverse a clinical condition (e.g. heparinisation).

These techniques involve the addition of the following reagents to the native whole blood sample:

Activators (celite, tissue factor, thrombin)

Heparin neutralisers (heparinase, protamine)

Platelet blockers (ReoPro, dipyridamole, ticlopine)

Antifibrinolytic drugs (epsilon-amino-caproic acid, tranexamic acid, aprotinin)

If native whole blood is used for analysis then the time between phlebotomy and starting the sample run must be less than 5 minutes. In circumstances when this is not possible sodium-citrate whole blood samples can be used. Citrated samples must be recalcified before analysis but give the investigator about 2 hours before the run must be commenced.

Temperature effect

TEG samples are generally run at 37°C. However, the apparatus does allow the investigator to change this and assess coagulation at other temperatures such as the use of hypothermia for severe head injuries.

Data analysis

The tracings can be analysed both qualitatively and quantitatively (Figs 8 and 9).

Quantitative data is readily transferable from the TEG software into commercial statistical packages such as Microsoft Excel and SPSS.

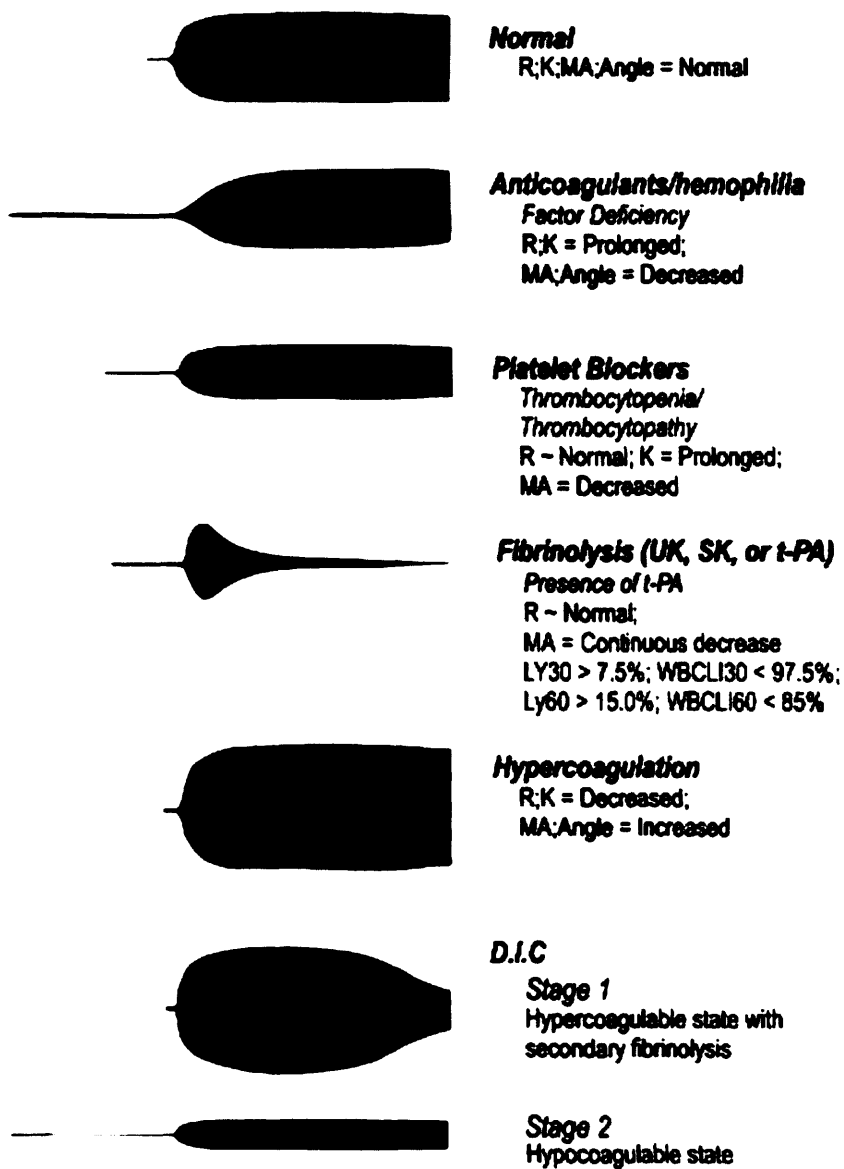


Figure 8. Qualitative TEG data(Mallett, Cox, 1992).

Mechanisms of haemostasis

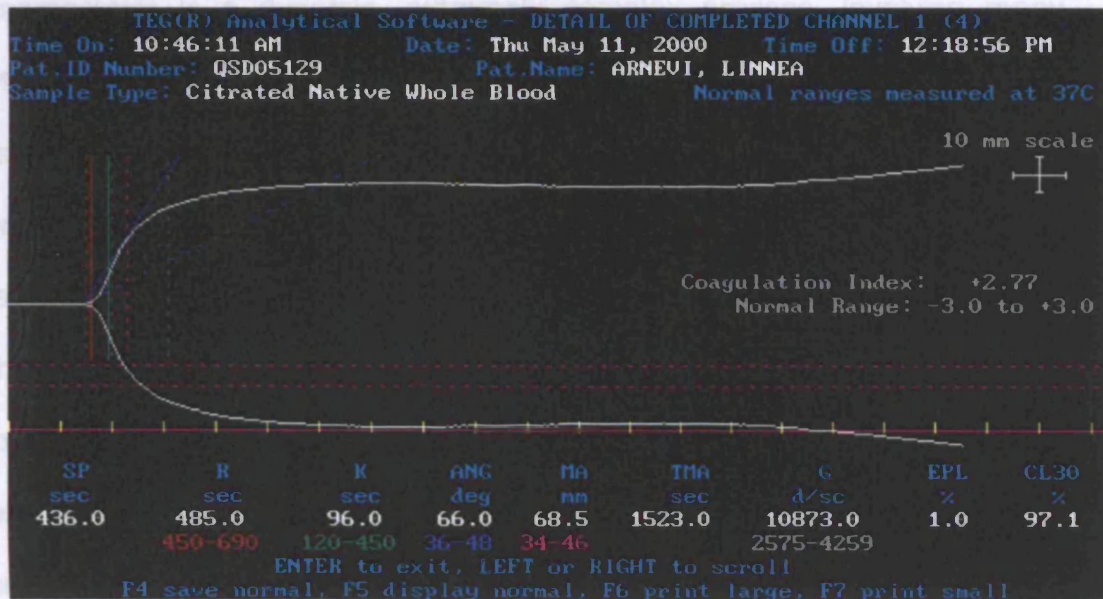


Figure 9. Quantitative TEG data.

A characteristic trace from a patient following a SAH. Each of the TEG parameters is generated automatically from the TEG software as the analysis proceeds.

enhance the structural rigidity of the fibrin network (Khurana et al., 1997).

Platelet GPIIb/IIIa receptors bind the polymerised fibrin network to the platelet actin cytoskeleton (Khurana et al., 1997). Through this receptor the platelets transmit a contractile force to the fibrin network (Fig. 10). It is the kinetics, strength and stability of this process that is measured by the TEG analyser. Each parameter represents a different aspect of the clot physical properties.

Mechanisms of haemostasis

Haemostasis is a dynamic, extremely complex process, involving many interacting factors, which include coagulation and fibrinolytic proteins, activators, inhibitors and cellular elements.

Once the coagulation cascade is activated, whether through the intrinsic pathway, the extrinsic pathway, or a combination of both, thrombin is formed. The thrombin cleaves soluble fibrinogen into fibrin monomers, which spontaneously polymerise to form protofibril strands that undergo linear extension, branching, and lateral association leading to the formation of a three dimensional network of fibrin fibres. A unique property of this network structure is that it behaves as a rigid elastic solid, capable of resisting deforming shear stress of flowing blood(Khurana et al., 1997).

Resistance to the deforming shear stress of the network of fibrin fibres is enhanced further by platelets, which are also activated by thrombin. Platelets achieve this in two ways. Firstly, they enhance fibrin polymerisation by acting as nodes or network branch points. Secondly, they stabilise and significantly enhance the structural rigidity of the fibrin network(Khurana et al., 1997).

Platelet GPIIb/IIIa receptors bind the polymerised fibrin network to the platelets actin cytoskeleton(Khurana et al., 1997). Through this receptor the platelets transmit a contractility force to the fibrin network (Fig.10). It is the kinetics, strength and stability of this process that is measured by the TEG analyser. Each parameter represents a different aspect of the clots physical properties.

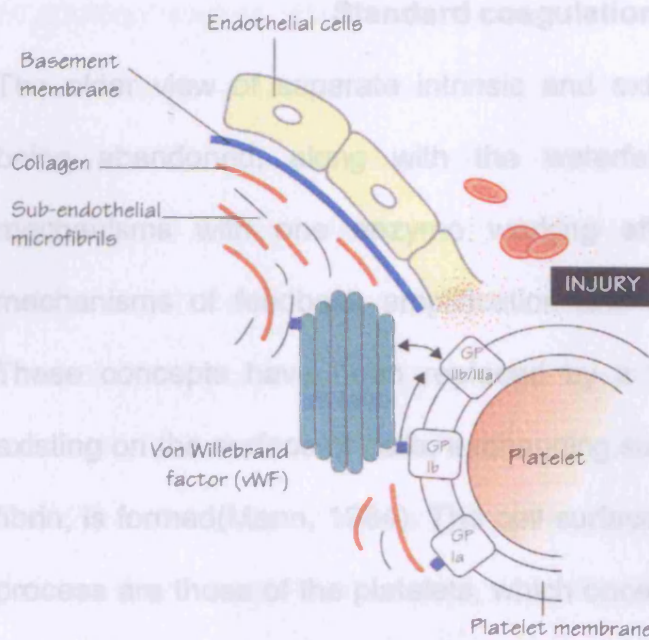


Figure 10. Platelet adhesion.

Subendothelial microfibrils bind vWF which in turn binds platelets at the glycoprotein 1b (GP1b) receptor. This binding exposes the platelet glycoprotein IIb/IIIa receptor which binds further with vWF. The GP IIb/IIIa receptor also binds fibrinogen to allow platelet-platelet aggregation. The platelet glycoprotein Ia receptor binds directly to collagen.

Taken from: Mehta, A and Hoffbrand V. Haematology at a Glance. 2000. Blackwell, Oxford, UK.

Standard coagulation tests

The older view of separate intrinsic and extrinsic coagulation systems is being abandoned, along with the waterfall description of coagulation mechanisms with one enzyme working after another, associated with mechanisms of feedback amplification and inhibition in a plasma milieu. These concepts have been replaced by a theory of enzyme complexes existing on the surface of cells, exchanging substrates until the final product, fibrin, is formed (Mann, 1984). The cell surfaces with most importance in this process are those of the platelets, which once activated adhere to the site of injury. They localise the clotting process, enhance their activity, and protect the enzyme complexes from the inhibitors that circulate to protect against the propagation of the clotting process downstream.

Since prothrombin time (PT) and activated partial thromboplastin time (APTT) are plasma tests designed with substitutes for platelet surfaces, it can be understood how these tests may not agree with whole blood TEG analysis. Haemostasis is a balance between the coagulation system forming fibrin and the fibrinolytic system breaking it down. Measuring isolated components of these systems ignores the interactive nature of the process. In addition quantitatively measuring a specific factor or protein gives no indication of its function in vivo (Miller et al., 1997). Functional activity is also dependent on the presence and activity of activators, inhibitors and cellular elements. Clotting is a dynamic process which is difficult to measure using stated end points, which provide no information about the quality of the clot or the dynamics of its formation (Mallett, Cox, 1992).

In addition, routine laboratory tests are generally performed on centrifuged plasma fractions and examine only isolated portions of the coagulation cascade, thereby overlooking important interactions essential to the clinical evaluation of clotting and bleeding syndromes(Mallett, Cox, 1992). Routine tests end with the formation of the first fibrin strands, whereas TEG begins at this point and continues to generate data as clotting continues through to eventual clot lysis or retraction(Spiess et al., 1987).

The PT is an in vitro measure of the extrinsic pathway and is prolonged in deficiencies/defects in factors II, V, VII and X, but is not affected by deficiencies in factors VIII, IX, and XI and the contact activation factors. The PT is performed by measuring the time it takes to form a clot after adding calcium and a tissue extract to plasma(Heesen et al., 1997b). The APTT measures activity of the intrinsic pathway and is prolonged by deficiencies/defects in factors II, V, IX, X, XI, and XII, but is not affected by deficiencies in factor VII. Both tests are sensitive to defects in the final common pathway. These tests do not exclude defects resulting from the vascular wall, platelet functional defects, some cases of von Willebrand disease or problems related to fibrinolysis.

Howland et al(Howland et al., 1974b) have compared TEG, PT and APPT for intraoperative coagulation monitoring in patients undergoing non-cardiac surgical procedures. The TEG provided information on hypercoagulability, platelet function, and fibrinolysis that other tests did not give, and it was concluded that TEG provided an extremely reliable guide for intraoperative therapy.

TEG provides an integrated profile of the whole blood haemostatic system. Earlier studies have shown a correlation between TEG variables and routine coagulation tests (platelet count, PT, APTT, antithrombin III, fibrinogen)(Artang et al., 2000; Zuckerman et al., 1981).

Endothelium

The inner lining of the whole circulatory system consists of a single layer of cells, the endothelium. In the capillaries an exchange of nutrients and hormones takes place facilitated by the relatively large exposure of endothelial surface area to a relatively small volume of blood. Endothelial cells from microvessels make a much larger contribution to circulating plasma factors that reflect endothelial functioning, than those from large vessels.

The endothelium contributes to the maintenance of blood fluidity by 3 groups of mechanisms. (A) It harbours factors that interrupt the coagulation cascade, such as antithrombin III, the protein C receptor thrombomodulin, and tissue factor pathway inhibitor. (B) It prevents platelet activation and aggregation by the production of nitric oxide and prostacyclin, exonucleotidases and surface heparan sulphates. (C) It can trigger and control fibrinolysis by the synthesis and release of tissue type plasminogen activator and its inhibitor PAI-1. The general properties of the endothelium are subject to adaptation by environmental factors, such as inflammatory mediators and shear forces. Interleukin-1 and tumour necrosis factor- α reduce the antithrombotic properties of the endothelium(van Hinsbergh, 2001).

Clinical applications of TEG

Thromboelastography (TEG) provides a comprehensive and permanent graphic documentation of the overall clotting process, from the formation of the first fibrin strands to clot dissolution. TEG measures the viscoelastic properties of the blood clot, and is said to be both objective and reproducible (Artang et al., 2000; Khurana et al., 1997; Mallett, Cox, 1992; Orlikowski et al., 1996; Spiess et al., 1987; Spiess et al., 1995).

TEG was developed first by Hartert in 1948 (Hartert, 1948). It remained largely a research tool until recent years, when the search for a quick, clinically useful 'bedside' test of coagulation rediscovered the value of TEG. Besides being useful in the detection of impaired coagulation, it has been suggested to be the most sensitive method for detecting a hypercoagulable state (Arcelus et al., 1995; Heather et al., 1980; Howland et al., 1974b; Howland et al., 1974a). Routine clotting studies are notoriously insensitive in this respect (Gibbs et al., 1994).

TEG has been used to demonstrate a hypercoagulable state in cancer patients (Raina et al., 1985), and a progressively hypercoagulable state in the first 7 days following open abdominal and laparoscopic surgery (Arcelus et al., 1995). A tendency to hypercoagulable TEG variables has been shown on the first postoperative day following laparoscopic cholecystectomy (Caprini et al., 1995). It has also been suggested that TEG can be used to predict the incidence of venous thromboembolism in high risk patients (Arcelus et al., 1995; Caprini et al., 1995; Heather et al., 1980; Pivalizza et al., 1997), and monitor the effects of anticoagulant medications (Arcelus et al., 1995; Gibbs, Sear, 1995; Gottlieb et al., 1999), in particular the effects of low dose heparin

therapy(Gibbs, Bell, 1998). TEG has been used to show maintenance of clot strength in stored and pooled blood platelets for transfusion(Reid et al., 1998).

Artang et al(Artang et al., 2000) demonstrated an increased procoagulant activity in TEG variables in ischaemic heart disease patients compared to healthy volunteers. However, the TEG did not hold enough diagnostic power to differentiate between non-coronary chest pain, unstable angina and acute myocardial infarction.

TEG has been used to suggest that extradural anaesthesia is superior to general anaesthesia when using lower or upper limb tourniquets in orthopaedic patients. These authors suggested that the increased coagulation associated with surgical/tourniquet pain was less with regional anaesthesia(Kohro et al., 1998).

In animal studies TEG has been used to demonstrate hypercoagulability induced by carcinoma(Raina et al., 1985).

TEG has been used to assess coagulation in pre-eclampsia(Orlikowski et al., 1991; Orlikowski et al., 1996) and the safety of epidural catheter placements in these women(James, Neil, 1995; Mallett, PLATT, 1991; Orlikowski et al., 1992; Sharma et al., 1999). These studies have shown the development of a hypercoagulable state during pregnancy which was not detected with other coagulation tests(Orlikowski et al., 1992). It has also been used to investigate the effect of local anaesthetics on coagulation(Porter et al., 1999).

TEG has shown a functional maturity of coagulation in neonates and children despite quantitative deficiencies in the coagulation system for the first 6

months of life(Miller et al., 1997). TEG has also been shown to have a high sensitivity and specificity in predicting neonatal sepsis where alterations in the vascular endothelium and thrombin generation are thought to underlie the pathophysiology(Grant, Hadley, 1997).

Handa and colleagues demonstrated it to be an effective screening test for prothrombotic states, thus preventing expensive screening tests in a significant proportion of these patients(Handa et al., 1997). Modified TEG using antiplatelet compounds has been used to determine the relative contributions of fibrinogen and platelets to clot strength(Gottumukkala et al., 1999).

However, by far the major clinical role identified at this time, has been in assessing the need for blood products (platelets, FFP, cryoprecipitate etc.), in actively bleeding surgical patients(Mallett, Cox, 1992; Oshita et al., 1999b; Spiess et al., 1987; Whitten, Greilich, 2000), or in patients with enhanced risk factors for a bleeding diatheses (Mongan, Hosking, 1992; Pivalizza, 1996; Pivalizza et al., 1996; Spiess et al., 1995). The ever increasing demands on blood banks for finite resources, together with the risk of transfusion related infection has led to a need to justify and give blood products in a directed manner. TEG directed therapy has been shown to reduce transfusion requirement in cardiopulmonary bypass patients without increasing post-operative haemorrhage(von Kier, Royston, 1998).

TEG is increasingly used in patients undergoing procedures with a significant haemorrhagic potential, such as liver transplantation and resection(Chapin et al., 1989; Howland et al., 1974a) and cardiopulmonary bypass surgery(Mongan, Hosking, 1992; von Kier, Royston, 1998). TEG appears to

be superior to routine coagulation tests in predicting peri and postoperative bleeding in patients undergoing liver transplantation and cardiac surgery(Artang et al., 2000; Essell et al., 1993; Spiess et al., 1987; Tuman et al., 1994).

TEG has previously found little use in the field of neurosurgery.

Procedure of TEG analysis used in all studies

The TEG[®] model 3000 analyser and computer system from the Haemoscope Corporation was used in all studies. All disposables and reagents are available through Haemoscope unless otherwise stated. Use of the TEG analyser is described in detail in the user manual: Thrombelastograph[®] Coagulation Analyser User Manual, Haemoscope Corporation.

Procedure

TEG analysis of citrated whole blood samples requires prior collection of 3.6 ml of blood into test tubes containing 0.105M (3.2%) buffered sodium citrate (Vacutainer[™] [Ref 367914]).

After mixing of blood and citrate by gentle inversion 3 times, samples are stored at room temperature. No further agitation of the sample takes place until immediately prior to analysis. TEG analysis should be commenced between 1 and 3 hours following blood sampling (Bowbrick et al., 2000; Camenzind et al., 1998).

Using a reverse pipette technique, 20 μ l 0.2M calcium chloride (CaCl_2) is dispensed into the cup of the analyser. A reverse pipette technique avoids the formation of air bubbles in the sample. The calcium chloride was placed in the cup first to prevent any surface activation of the blood sample without recording, which might occur if performed in reverse order.

Immediately prior to analysis, each citrated whole blood sample is mixed by gently inverting the tube 5 times. Then 340 μ l is then dispensed into the cup. Mixing of the blood and CaCl_2 is achieved by 5 cycles of gentle lowering and raising the pin column. The pin is then left in the sample and automated TEG

analysis commenced via a computer interface (TEG[®] Analytic Software Version 2.99.70x, Haemoscope Corporation 1995).

After starting the analysis, a drop of mineral oil (Haemoscope Ref. No. #302-895) is placed on the surface of the sample. This prevents artefacts secondary to evaporation and drying of the sample.

Quality control

Daily assessment of the analyser balance, baseline and operating temperature was performed.

Monthly calibration of the analyser using Level I and Level II coagulation controls (Haemoscope Corporation, Skokie, IL, USA) was carried out.

Precautions

Standard safety precautions whilst handling blood samples were taken at all times.

Some common pitfalls

Difficult or slow collection during blood sampling.

Heparin contamination when sampling through arterial lines.

Addition of an incorrect volume of blood to the citrated test tube.

Delay in mixing blood with citrated anticoagulant.

Prolonged or sub-optimal blood storage prior to analysis.

Manufacturer details

Haemoscope Corporation

7855 Gross Point Road, Unit G-4,

Skokie, IL, 60077 USA

www.haemoscope.com

Control studies

Although the TEG proprietary software generates a reference range of 'normal' values for a specific sample type, it is unclear how these ranges were collected, and on what population of people/patients. There is little published control data for TEG, and what has been published suggests that TEG variables are sensitive to blood sampling techniques and sample handling.

I have assessed the reproducibility of TEG from a single sample and assessed its reproducibility over time. Its stability over time was central to the clinical studies as I went on to see how TEG profiles changed over time following a subarachnoid haemorrhage (SAH).

I have produced reference values for the TEG variables in a control population using the sampling techniques and sample handling methods employed in the clinical studies. I have assessed the effects of age and gender on TEG variables. The reference ranges generated by sample type from the TEG software make no distinction for age or gender, suggesting that there is no difference. Stability with regard to gender was important to the clinical studies on SAH as this condition has a distinct female preponderance.

Control study 1

The primary aim of this study was to assess the reproducibility of a TEG tracing from a single citrated blood sample in a control population i.e. that the machine was a reproducible measure of coagulation.

Control study 2

The primary aim of this study was to ascertain the ranges for TEG variables in a control population.

The secondary aim of this study was to assess the effect of gender and age on haemostatic parameters as measured by TEG in a control population.

Control study 3

The primary aim of this study is to ascertain the reproducibility of TEG over time in a control population.

Control study 1

The primary aim of this study was to assess the reproducibility of a TEG tracing from a single citrated blood sample in a control population.

Method

For this study 20 subjects were enrolled, 10 male (mean age 31.4 yrs SD 5.1) and 10 female (mean age 30.6 yrs SD 10.9).

Citrated whole blood was sampled from each subject and each sample run simultaneously through 2 separate TEG channels.

Sampling

Peripheral venous blood from an antecubital vein was sampled through a 19-gauge butterfly needle into a 3.6 ml 'blue-capped' Vacutainer polypropylene tube, containing 0.5 ml of 3.2% (0.105M) sodium citrate (pH 7.4) (Vacutainer™ [Ref 367914]). Smaller gauge needles were avoided as they precipitate haemolysis and activate clotting mechanisms.

Whenever possible, blood samples were obtained without the use of a tourniquet, to reduce the likelihood of in vitro coagulation activation (Miller et al., 1995). If a tourniquet was used, it was applied for venous access only, and it was removed as blood was collected.

The tube was then inverted 3 times in order to mix the blood and citrate immediately after sampling. Care must be taken not to shake the sample violently as this may itself activate coagulation. This citrated whole blood sample (CWB) was then left to stand for 60 minutes at room temperature.

It is important to standardise storage time as it has been previously demonstrated that some TEG parameters can decrease with time, perhaps due to a decrease in fibrinogen and platelets with storage in citrated

tubes(Willschke et al., 1999). Others have observed a tendency to hypercoagulation in the first 30 minutes of storage(Camenzind et al., 1998). Blood collection and handling is a major source of bias in haemostatic analysis. Ex-vivo 'contact' activation of the coagulation cascade cannot be completely avoided and must be limited to a minimum by standardised blood sampling. Poor venepuncture or prolonged venous occlusion may result in an increase of haemostatic reactions including thrombin and plasmin activity(Winkler, 1996).

Blood sampling and TEG analysis was carried out in a standard manner for each control study.

Exclusions

Any person, who had a known coagulation disorder, was taking anticoagulant medication, had taken a non-steroidal anti-inflammatory medication in the preceding 2 weeks, or was taking the oral contraceptive pill, was excluded from participation in the study. Pregnant and paediatric (<18 yrs) subjects were also excluded.

Consent

All control studies were voluntary and verbal consent was taken from each participant. Participants were instructed in the aims, procedures and methods involved in the study. Any participant could withdraw from the study at any time. No participants received remuneration for their involvement in the study.

Ethical approval

All studies were approved by the joint medical ethics committee of the Institute of Neurology and University College Hospitals Trust (**Ref. No. 99/N037**)

Statistical analysis

The mean values for each TEG parameter were calculated, along with its standard deviation in order to give an indication of the reference range of each variable in a control population. Since 2 readings were available for each sample, the mean difference between samples, with its standard deviation and 95% confidence interval is presented.

An estimate of within person standard deviation was calculated from the formula:

$$\text{Mean of the difference } X1-X2 \div \sqrt{2}$$

The coefficient of variance for each TEG variable was calculated from the formula:

$$(\text{Mean of the difference } X1-X2 \div \sqrt{2}) \div \text{Mean of total population} \times 100$$

Results

R time

Since TEG analysis was performed twice on each sample, 2 values for R were available for each individual, R1 and R2. The mean value for R in all subjects was 639.15 s, with a standard deviation of 90.88 s. The mean difference between the two measurements of R (R1-R2) was 21.4 s, with a standard deviation of 46.68 s. The 95% confidence limits were 0.96 to 41.86 s (Table 1).

The estimate of within person standard deviation for R time was 15.15 s. The coefficient of variation was 2.37% (Table 2).

K time

The mean K time for the population was 159.65 s (SD 90.88). The mean difference K1-K2 was -3.1 s (SD 12.42). The 95% confidence limits were -8.54 to 2.34 s (Table 1).

The estimate of within person standard deviation for K time was 2.19 s. The coefficient of variation was 1.37% (Table 2).

Angle

The mean angle for the population was 55.24 deg (SD 7.23). The mean difference Angle1-Angle2 was -0.38 deg (SD 2.24). The 95% confidence limits were -1.36 to 0.6 deg (Table 1).

The estimate of within person standard deviation for Angle was 0.27 deg. The coefficient of variation was 0.49% (Table 2).

MA

The mean MA for the population was 52.15 mm (SD 4.2). The mean difference MA1-MA2 was 1.45 mm (SD 3.1). The 95% confidence limits were 0.29 to 2.81 mm (Table 1).

The estimate of within person standard deviation for MA was 1.03 mm. The coefficient of variation was 1.97% (Table 2).

Shear elastic modulus G

The mean G value for the population was 5538.58 dyn/cm² (SD 932.43). The mean difference G1-G2 was 348.5 dyn/cm² (SD 614.77). The 95% confidence limits were 79.07 to 617.93 dyn/cm² (Table 1).

The estimate of within person standard deviation for G was 246.43 dyn/cm².

The coefficient of variation was 4.45% (Table 2).

Discussion

	Mean	SD	95% CI
R s	639.15	90.88	39.82
R1 - R2 s	21.4	46.68	20.46
K s	159.65	45.04	19.74
K1 - K2 s	-3.1	12.42	5.44
Angle deg	55.24	7.23	3.17
A1 - A2 deg	-0.38	2.24	0.98
MA mm	55.15	4.2	1.84
MA1 - MA2 mm	1.45	3.1	1.36
G dyn/cm2	5538.58	932.43	408.65
G1 - G2 dyn/cm2	348.5	614.77	269.43

Table 1. Summary statistics of TEG variables in control study 1.

SD = Standard deviation, CI = Confidence interval.

	R	K	Angle	MA	G
Estimate of within person SD	15.13	2.19	0.27	1.03	246.4
Coefficient of variation %	2.37	1.37	0.49	1.97	4.45

Table 2. Summary statistics of agreement between X1 and X2 in each of the 20 subjects for the TEG variables R, K, Angle, MA and G.

Discussion

The interassay coefficient of variation of the APTT test, calculated on a normal plasma sample repeated 12 times is 3.5%(Fourel et al., 1993).

For tissue factor stimulated citrated whole blood TEG analysis, Khurana et al showed an intrasubject coefficient of variation to be 1.4% +/- 0.2% for MA and 4.4%+/- 1.3% for G when TEG analysis was repeated 8 times on 8 separate subjects. The intraclass correlation coefficient was $r=0.96$ (Khurana et al., 1997).

Surprisingly, there is no other published study to address the subject of the reproducibility of TEG on a given blood sample. No control data is available from the manufacturers (Haemoscope Corp.,Skokie, IL, USA).

My results suggest that the TEG is a highly reproducible measurement, and is at the very least comparable to APPT(Fourel et al., 1993). The K time, Angle and MA provide the most reproducible measurement. As G is a derived variable from MA in order to exponentially enhance the measurement [$G = 5000 \times MA + (100 - MA)$], it is not surprising that it has the greatest coefficient of variation, and is the least reproducible measure.

The reproducibility of the TEG is demonstrated in Figs 11 to 15. The value of each TEG parameter X1 is plotted against the second measurement X2. If the measurements had agreed exactly then they would all lie on the illustrated line of equality.

Conclusion

TEG is a reproducible measurement of coagulation on a single citrated blood sample.

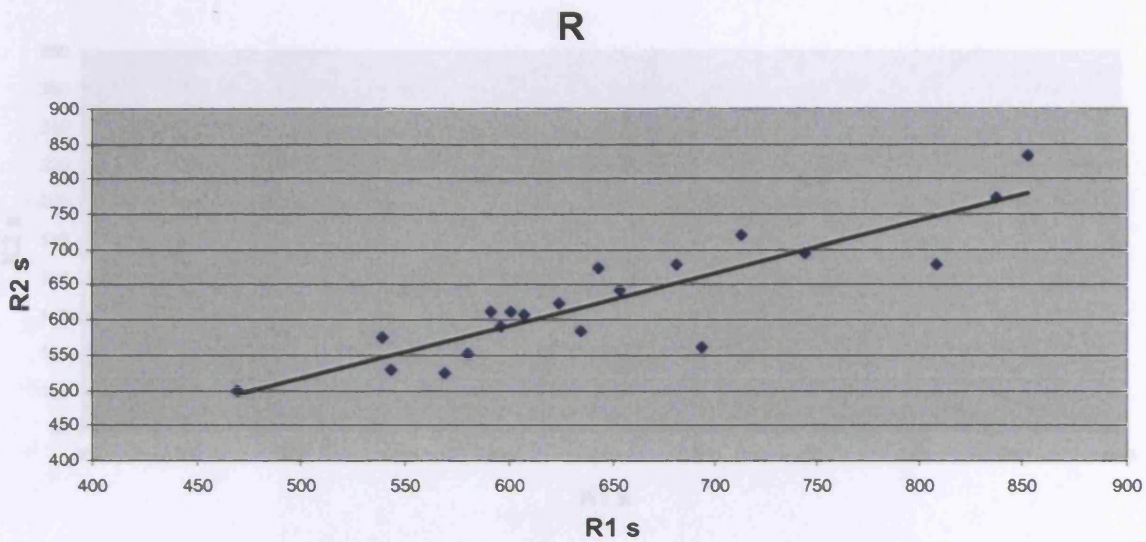


Figure 11. Scatter plot of R1 and R2.

Figure 11. Scatter plot of R1 against R2.

Scatter diagram of the first series of measurements for R plotted against the second series in the 20 normal controls. Had all the measurements agreed exactly, the points would all lie on the line of equality. All data points are close, indicating a good reproducibility of the test.

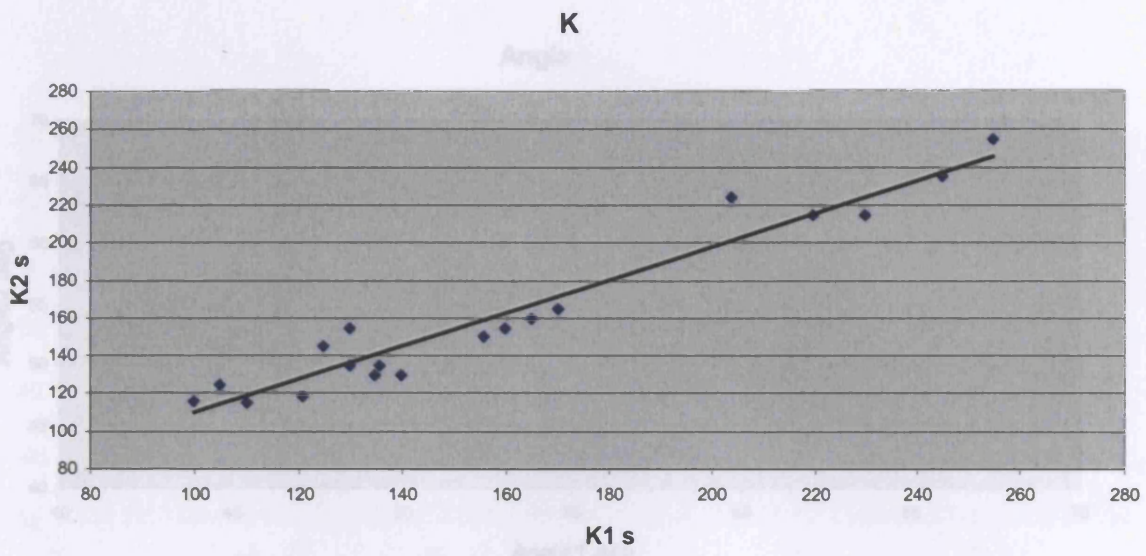


Figure 12. Scatter plot of K1 and K2.

Figure 13. Scatter plot of Angle1 and Angle2.

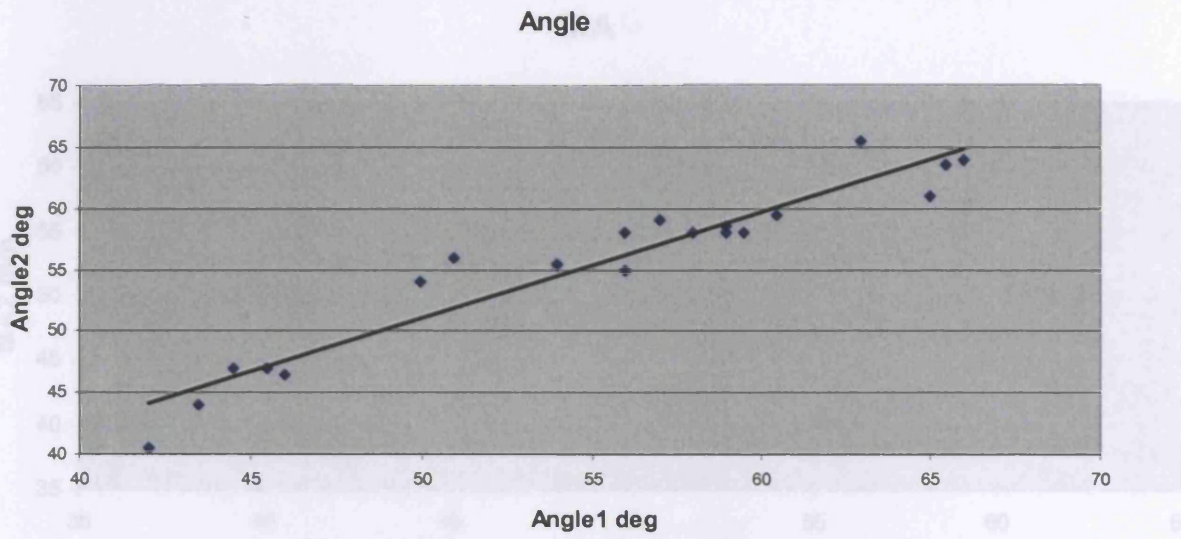


Figure 13. Scatter plot of Angle1 and Angle2.

Figure 14. Scatter plot of MA1 and MA2.

MA

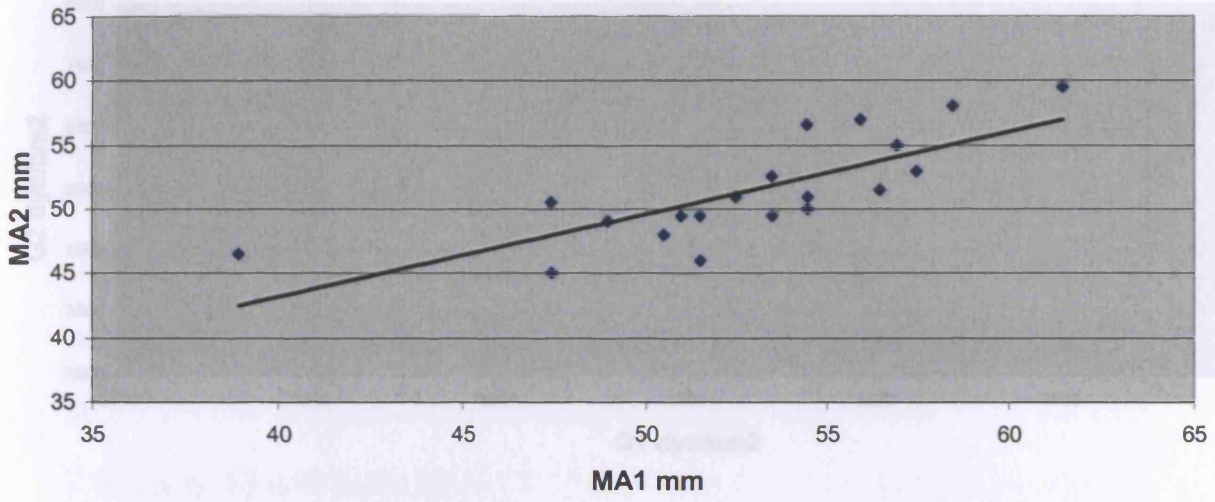


Figure 14. Scatter plot of MA1 and MA2.

Control study 2

The primary aim of this study was to ascertain ranges for TEG variables in a control population.

G

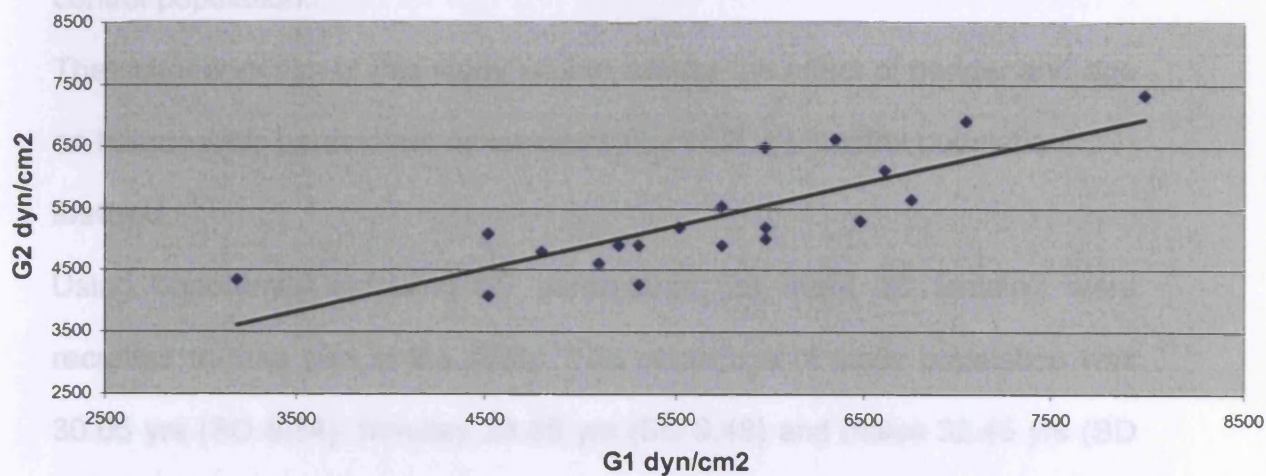


Figure 15. Scatter plot of G1 and G2.

Results

The mean value of R was 574.7 s (SD 141.3) and 95% confidence interval of +/- 43.8 s. The mean value of K was 176.4 s (SD 52.3) and 95% confidence interval of +/- 16.2 s. The mean value of the Angle was 52.95 deg (SD 7.8) and 95% confidence interval of +/- 2.4 deg. The mean value for MA was 53.4 mm (SD 4.1) and 95% confidence interval of +/- 1.3 mm. The mean value for G was 5751.8 dyn/cm2 (SD 997.3) and 95% confidence interval of +/- 309.1

Control study 2

The primary aim of this study was to ascertain ranges for TEG variables in a control population.

The secondary aim of this study was to assess the effect of gender and age on haemostatic parameters as measured by TEG in a control population.

Method

Using opportunity sampling 40 participants (20 male, 20 female), were recruited to take part in the study. The mean age of study population was 30.65 yrs (SD 8.84): females 28.85 yrs (SD 9.48) and males 32.45 yrs (SD 7.97) (Fig. 16 and Table 3).

Sampling as described in control study 1.

Exclusions as for control study 1.

Statistical analysis

The mean, standard deviation and 95% confidence intervals for each of the TEG variables, was calculated for the population of 40 subjects.

Using Microsoft Excel software, an ANOVA regression analysis was performed on each of the TEG parameters (R, K, Angle, MA and G) for age and sex in each of the 40 subjects.

Results

The mean value of R was 574.7 s (SD 141.3) and 95% confidence interval of +/- 43.8 s. The mean value of K was 175.4 s (SD 52.3) and 95% confidence interval of +/- 16.2 s. The mean value of the Angle was 52.95 deg (SD 7.8) and 95% confidence interval of +/- 2.4 deg. The mean value for MA was 53.4 mm (SD 4.1) and 95% confidence interval of +/- 1.3 mm. The mean value for G was 5791.8 dyn/cm² (SD 997.3) and 95% confidence interval of +/- 309.1

dyn/cm². This gives an indication of the ranges of these variables in a control population using the sampling methods and exclusions described (Table 4).

When R was regressed for age and sex there was no statistically significant difference identified $F(2,39) = 2.76$; $p > 0.05$ (Tables 5, 10 and 11, and Figure 17). However, for each of the other TEG parameters there was a statistically significant trend towards hypercoagulability in the female controls when compared with the males. There was no association between the age of the control subject and any TEG variable studied (Tables 5 to 9).

Thus, the mean K time was significantly shorter in female than in male controls $F(2,39) = 8.55$; $p < 0.001$ (Tables 6 and 10). The angle was greater in females $F(2,39) = 8.92$; $p < 0.001$ (Tables 7, 10 and 12, and Figure 18). The MA was greater in females $F(2,39) = 10.02$; $p < 0.001$ (Tables 8 and 10). And the value for G was on average higher in females $F(2,39) = 8.22$; $p < 0.001$ (Tables 9, 10 and 13, and Figure 19).

These results demonstrate female controls lean towards hypercoagulability when compared with males. This difference could not be explained by a difference in age between the sample populations.

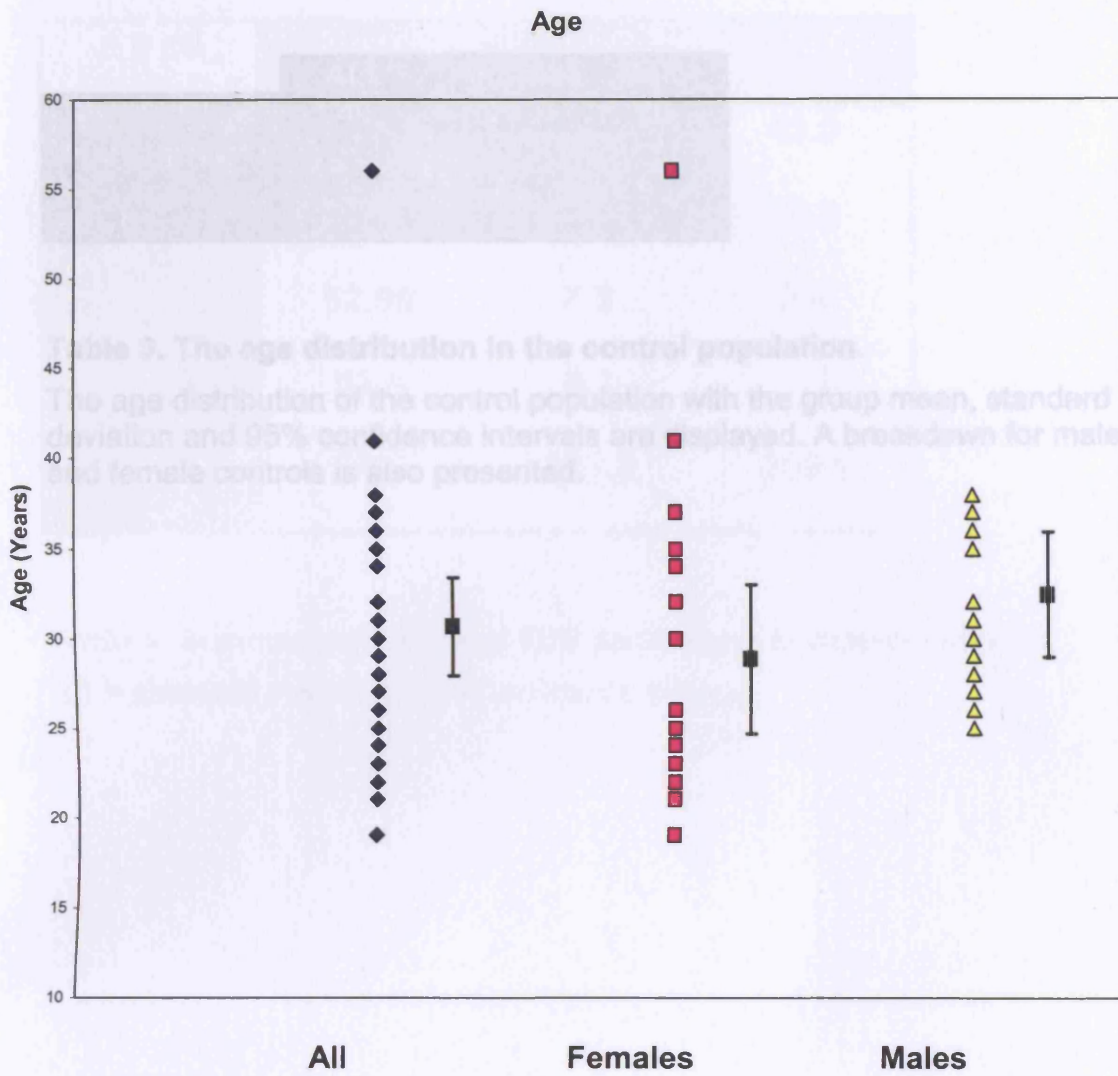


Figure 16. Scatter diagram of the age distribution in the control population.

The individual values, group mean and 95% confidence intervals are displayed. A breakdown for male and female controls is also presented.

	ALL	Female	Male	95% CI
<i>n</i>	40	20	20	
Mean age (yrs)	30.65	28.8	32.45	43.8
SD	8.84	9.48	7.97	18.2
95% CI	2.74	4.16	3.49	

Table 3. The age distribution in the control population.

The age distribution of the control population with the group mean, standard deviation and 95% confidence intervals are displayed. A breakdown for male and female controls is also presented.

Table 4. Summary statistics of TEG parameters in control subjects.
SD = standard deviation, CI = Confidence Interval

n = 40	Mean	SD	95% CI
R	574.7	141.3	43.8
K	175.4	52.3	16.2
Angle	52.95	7.8	2.4
MA	53.4	4.1	1.3
G	5791	997.3	309.1

Table 4. Summary statistics of TEG parameters in control subjects.

SD = standard deviation, CI = Confidence interval

Table 5. Analysis of the effects of gender and age on R in controls.

Using $p < 0.05$ to determine significance, regression analysis of R for effects of gender and age revealed that neither age nor gender produced a significant effect on R ($p > 0.05$).

Regression Statistics	
Multiple R	0.36
R Square	0.13
Adjusted R Square	0.08
Standard Error	135.30
Observations	40

ANOVA

	df	SS	MS	F	Significance F
Regression	2	100896.37	50448.19	2.76	0.08
Residual	37	677326.4	18306.11		
Total	39	778222.78			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	374.40	92.63	4.04	0.00	186.72	562.08
sex	81.24	43.73	1.86	0.07	-7.36	169.84
age	2.56	2.51	1.02	0.31	-2.52	7.64

Table 5. Analysis of the effects of gender and age on R in controls.

Using $p < 0.05$ to determine significance, regression analysis of R for effects of gender and age revealed that neither age nor gender produced a significant effect on R ($p > 0.05$).

Regression Statistics	
Multiple R	0.56
R Square	0.32
Adjusted R Square	0.28
Standard Error	44.42
Observations	40

ANOVA

	df	SS	MS	F	Significance F
Regression	2	33738.69	16869.34	8.55	0.0008
Residual	37	73004.91	1973.11		
Total	39	106743.6			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	93.05	30.41	3.06	0.004	31.43	154.66
sex	58.66	14.36	4.09	0.0002	29.58	87.75
age	-0.18	0.82	-0.22	0.82	-1.85	1.48

Table 6. Analysis of the effects of gender and age on K in controls.

Using $p < 0.05$ to determine significance, regression analysis of K for effects of gender and age revealed that gender (but not age) produced a significant effect on K ($p < 0.001$).

Regression Statistics	
Multiple R	0.57
R Square	0.33
Adjusted R Square	0.29
Standard Error	6.61
Observations	40

ANOVA

	df	SS	MS	F	Significance F
Regression	2	778.87	389.43	8.92	0.0007
Residual	37	1614.53	43.64		
Total	39	2393.4			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	65.16	4.52	14.41	9.40E-17	56.00	74.32
sex	-8.94	2.13	-4.19	0.0002	-13.27	-4.62
age	0.04	0.12	0.32	0.75	-0.21	0.29

Table 7. Analysis of the effects of gender and age on Angle in controls.

Using $p < 0.05$ to determine significance, regression analysis of Angle for effects of gender and age revealed that gender (but not age) produced a significant effect on Angle ($p < 0.001$).

Regression Statistics	
Multiple R	0.59
R Square	0.35
Adjusted R Square	0.32
Standard Error	3.36
Observations	40

ANOVA

	df	SS	MS	F	Significance F
Regression	2	226.59	113.30	10.02	0.0003
Residual	37	418.51	11.31		
Total	39	645.1			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	59.33	2.30	25.77	3.096E-25	54.67	64.00
sex	-4.84	1.09	-4.47	7.14E-05	-7.06	-2.66
age	0.04	0.06	0.71	0.48	-0.08	0.17

Table 8. Analysis of the effects of gender and age on MA in controls.

Using $p < 0.05$ to determine significance, regression analysis of MA for effects of gender and age revealed that gender (but not age) produced a significant effect on MA ($p < 0.001$).

Regression Statistics	
Multiple R	0.55
R Square	0.31
Adjusted R Square	0.27
Standard Error	852.00
Observations	40

ANOVA

	df	SS	MS	F	Significance F
Regression	2	11933878	5966939	8.22	0.001
Residual	37	26858648	725909.41		
Total	39	38792526			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	7092.02	583.28	12.16	1.725E-14	5910.18	8273.9
sex	-1116.3	275.35	-4.05	0.0002	-1674.21	-558.39
age	12.21	15.78	0.77	0.44	-19.76	44.18

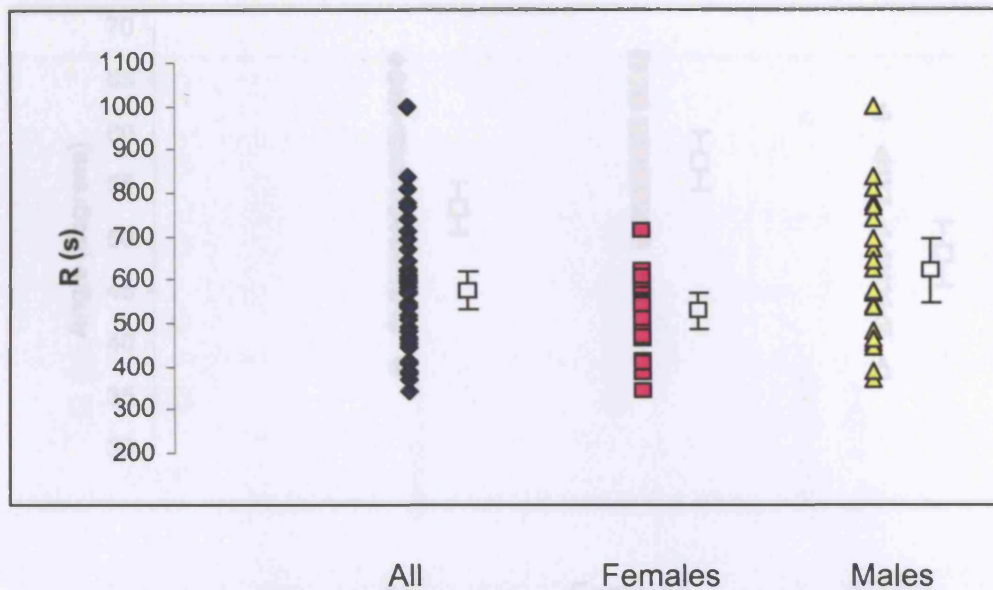
Table 9. Analysis of the effects of gender and age on G in controls.

Using $p < 0.05$ to determine significance, regression analysis of G for effects of gender and age revealed that gender (but not age) produced a significant effect on G ($p < 0.001$).

	N	Female Mean (SD)	Male Mean (SD)	F	P-value
R	40	529.45 (94.25)	619.90 (166.64)	2.76	0.07
K	40	146.40 (36.56)	204.40 (50.11)	8.55	0.0002
Angle	40	57.35 (2.25)	48.55 (6.81)	8.92	0.0002
MA	40	55.75 (2.25)	51.05 (4.15)	10.2	7.14E-05
G	40	6327.98 (591.96)	5255.63 (1042.15)	8.22	0.0002

Table 10. Summary table showing the F and P values for the regression analysis of gender on all TEG variables in controls.

SD = Standard Deviation.



	Mean	Standard Deviation	Confidence Interval
All	574.68	141.26	43.78
Females	529.45	94.25	41.31
Males	619.9	166.64	73.03

Figure 16. Scatter diagram of Angle in control subjects.

There was a significant difference between males and females for TEG parameter Angle ($p < 0.001$).

Figure 17. Scatter diagram of R in control subjects.

There was no significant difference between males and females for TEG parameter R ($p > 0.05$).

Table 11. Summary table of R in control subjects.

Table showing the mean, SD and CI for All subjects, female subjects and male subjects for TEG variable R.

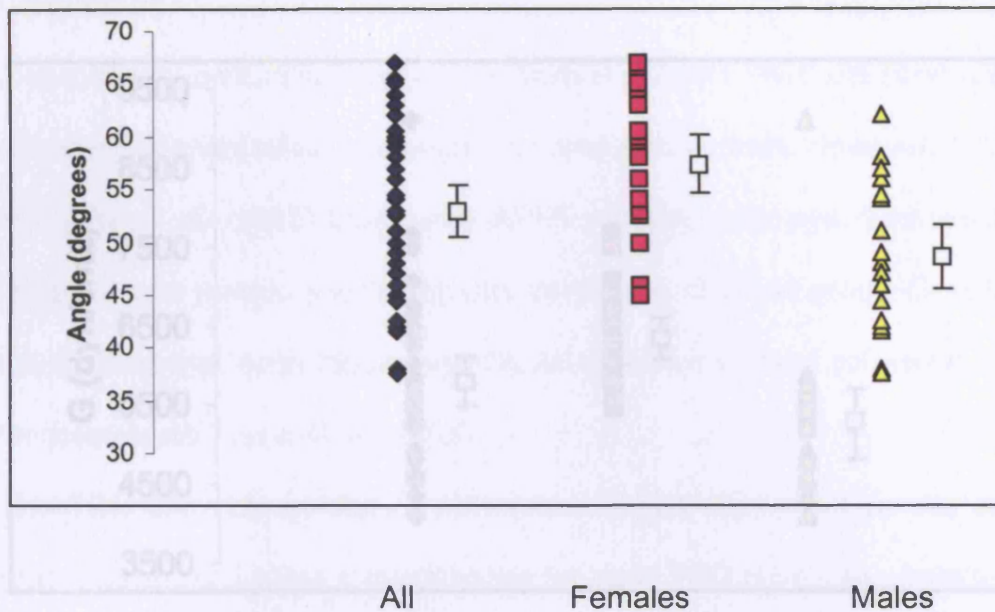
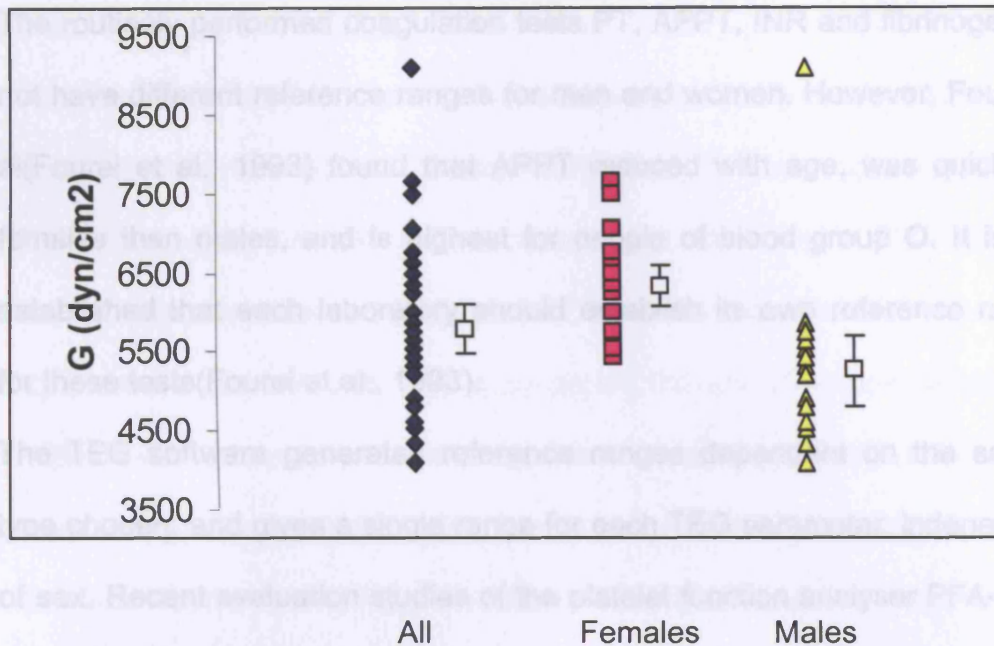


Figure 18. Scatter diagram of Angle in control subjects.

There was a significant difference between males and females for TEG parameter Angle ($p < 0.001$).

Table 12. Summary table of Angle in control subjects.

Table showing the mean, SD and CI for All subjects, female subjects and male subjects for TEG variable Angle.



	Mean	Standard Deviation	Confidence Interval
All	5791.8	997.34	309.67
Females	6327.98	591.96	259.43
Males	5255.36	1042.15	456.13

Figure 19. Scatter diagram of G in control subjects.

There was a significant difference between males and females for TEG parameter G ($p < 0.001$).

Table 13. Summary table of G in control subjects.

Table showing the mean, SD and CI for All subjects, female subjects and male subjects for TEG variable G.

Discussion

The routinely performed coagulation tests PT, APPT, INR and fibrinogen, do not have different reference ranges for men and women. However, Fourel et al(Fourel et al., 1993) found that APPT reduced with age, was quicker in females than males, and is highest for people of blood group O. It is well established that each laboratory should establish its own reference ranges for these tests(Fourel et al., 1993).

The TEG software generates reference ranges dependent on the sample type chosen, and gives a single range for each TEG parameter, independent of sex. Recent evaluation studies of the platelet function analyser PFA-100[®], have failed to identify any difference in platelet function related to sex or OCP use(Bock et al., 1999).

Most control and study TEG populations have not allowed for any sex difference in coagulation variables and are not controlled for this variable. Indeed, Procidano and co-workers suggested in a study on 777 subjects (271 males, 506 females) that neither age nor gender had any influence on TEG variables(Prodicano et al., 1983).

However, Gorton et al(Gorton et al., 2000) have recently used TEG to suggest that women have more whole blood coagulability than men. They demonstrated an increasing trend through men, nonpregnant women to pregnant women. It has been suggested that the physiology of pregnancy results in a hypercoagulable state that prepares the parturient for the haemorrhage of childbirth(Whitten, Greilich, 2000). Pregnant women have also been shown to have higher TAT and PF 1 and 2 complexes, than age

matched non-pregnant controls. These complexes were shown to increase with advancing gestational age(Levine et al., 1996).

The data reported by Gorton et al was compromised by the fact that half of the 50 nonpregnant women studied were taking the oral contraceptive pill (OCP). Interestingly however, they reported no difference in TEG variables between those volunteers taking the OCP and those who were not.

Gorton et al argued that their data supported the sex differences in markers of thrombotic tendency observed by Lowe et al(Lowe et al., 1997). They concluded that endogenous hormones may have a roll in sex-related differences in the incidence of deep vein thrombosis and thromboembolism.

Lowe et al(Lowe et al., 1997) had randomly sampled 1958 men and women aged 25-75 yrs as part of the third MONICA survey in Glasgow, Scotland.

They showed that pre-menopausal women generally had higher levels of fibrinogen and prothrombin F1 and 2, and lower levels of Factor IX, and protein S than men. Post-menopausal women had higher levels of fibrinogen, Factor VII and Factor IX than pre-menopausal women.

Clues as to the effects that sex hormones may play on coagulation responses can be ascertained by looking at the changes observed following the introduction of the oral contraceptive pill. The use of oral contraceptives is associated with altered plasma concentrations of many components of the coagulation and fibrinolytic systems, increased plasma levels of markers indicating in vivo coagulation and fibrinolysis, and a modified response to challenge tests both in vivo and in vitro(Levine et al., 1996; Winkler, 1998).

Differences in fibrinogen, thrombin-antithrombin (TAT), prothrombin fragment 1 and 2, antithrombin III, protein C, protein S, tissue plasminogen activator,

plasminogen and plasminogen activator inhibitor have all been observed after starting combined oral contraceptive preparations(Prasad et al., 1999). Though in this study a dynamic balance between coagulation and fibrinolysis was observed with no predisposition to thrombosis after starting the preparations. However, other studies have suggested that in women taking the oral contraceptive pill, an increase in the activity of the procoagulant Factor VII, Factor X, and fibrinogen occurs, and a fall in anticoagulant antithrombin III may predominate(1999a). Further studies have shown that the effect on coagulation factors is highly dependant on the hormone combination used(Dykes et al., 2001; Mackie et al., 2001; Viegas et al., 1996; Winkler et al., 1998) and the route of administration(Bulent-Tiras et al., 2001; Viegas et al., 1996). The use of ethinylestradiol is associated with an increase of plasma concentrations of fibrinogen, plasminogen, Factors VII, VIII, X and XIII, but also coagulation inhibitors such as protein C(Winkler, 1996). Progesterone only contraceptive pills produce little or much reduced haemostatic alteration(Winkler et al., 1998).

Following the introduction of the oral contraceptive pill the 1960's, it became recognised that venous thrombosis and pulmonary emboli and later myocardial infarction and stroke were associated with its use(Rosing et al., 1999; Vandenbroucke et al., 2001). The newer, lower dose oestrogen preparations were considered safer, with the changes in haemostatic factors smaller, inconsistent in direction and mostly within the normal range.

However, recent studies have challenged the concept that reducing the dose of oestrogen in oral contraceptives eliminates the risk of venous thrombosis. These studies have suggested that progestagens, individual genetic

susceptibility to the thrombogenic effect of oral contraceptives, and new insights into haemostatic changes in women, may all play a role in the risk of thrombosis(Vandenbroucke et al., 2001). It has been suggested that exogenous hormone administration results in an acquired resistance to protein C, which may underlie its prothrombotic tendency(Rosing et al., 1999). This resistance appears dependent on the particular OCP formula used, in particular the progestagen used(Rosing et al., 2001).

Arterial thrombosis is also a complication of oral contraceptive therapy, but interestingly, the risk factors differ from those for venous thrombosis. For example, smoking increases the risk of myocardial infarction associated with the use of oral contraceptives, but has no material effect on the risk of venous thrombosis. In contrast, several prothrombotic genetic defects are strong risk factors for venous thrombosis and increase the risk associated with oral contraceptive use(Girolami et al., 2001), but most are likely to be only weak risk factors for myocardial infarction or stroke(Vandenbroucke et al., 2001).

Little clinical work has been directed at coagulation differences between the sexes. However, stroke incidence is higher in young women than in young men between the ages of 15 and 35 years and there is a significant association of stroke with pregnancy(Jaigobin, Silver, 2000). Jaigobin reported an incidence for stroke both ischaemic (arterial and venous) and haemorrhagic of 69 strokes per 100000 during pregnancy. This compares with an incidence in the population as a whole of 3.5 per 100000 for ischaemic stroke. While there were reasons to explain some of this dramatic difference in terms of the aetiology of the incident population studied, there is

consensus that the incidence of all stroke forms appears higher during pregnancy. It is thought that the risk is greatest in the postpartum period. Aetiological factors include eclampsia and pre-eclampsia, coagulopathy, hormonal and blood volume changes, and dehydration. Most studies have also reported a decrease in both thromboembolic and haemorrhagic stroke in women using HRT (Brighthouse, 2001).

Kalaria et al (Kalaria et al., 2000) looked at gender related differences in thrombogenic factors predicting recurrent cardiac events in patients after acute myocardial infarction. They measured multiple factors in 791 men and 254 women, 2 months following infarction. In univariate analysis of haemostatic factors, levels of d-dimer, Factor VII, activated Factor VII (VIIa), fibrinogen, and von Willebrand factor were significantly higher in women. In multivariate logistic regression, fibrinogen, Factor VII and VIIa remained significantly higher. In addition, elevated Factor VIIa levels were a significant predictor of further cardiac events in women. The authors propose an enhanced thrombogenic state in women compared with men following acute myocardial infarction. They suggest genetic differences, sex hormones and environmental factors such as obesity, smoking and lipid levels, as possible explanations for their findings.

Until recently, it had never previously been suggested that there were differences in TEG variables between male and female controls. The results of this study show similar findings to those of Gorton et al (Gorton et al., 2000), in that females were hypercoagulable in comparison to males. I have shown that these findings were not a product of a difference in ages between the 2 populations studied.

It is interesting to note that the only TEG parameter in which I have failed to demonstrate a difference in male and female controls is the R time. This is contrary to the findings of Gorton et al (Gorton et al., 2000) who did observe a significant difference in the R time. It has been suggested that the R time is comparable to the PT and APPT, as it is the time point at which the first fibrin strands start to form. Thus, it appears that the first detectable signs of clot formation are identified at the same time in males and females. This data also suggests that the routine tests PT and APPT would not have picked up the sex differences in coagulation profiles identified with TEG.

The additional TEG variables (K, Angle, MA and G) are said to give information not available from routine coagulation studies. The K time was shorter and the Angle greater in females. This suggests that once a clot starts to form, the rate of fibrin build up and cross-linking is greater in females.

The observed changes in MA and G show that females on average have a higher maximal value. MA and G are directly dependent on fibrinogen and platelet function. These results suggest that the final clot strength is greater in female controls, than in age matched males.

The results suggest that in the control population studied, females tend towards hypercoagulability. This could be linked to the fact that women have naturally lower concentrations of the natural anticoagulants (protein C and protein S)(Dykes et al., 2001), and higher concentrations of procoagulants (factor VIII)(Lowe et al., 1997; Woodward et al., 1997).

Kapiotis et al(Kapiotis et al., 1998) found that Factor VII levels changed during the menstrual cycle and they argued that this supported the idea that coagulation factors may be up or down regulated by sex hormones.

Mendelsohn et al(Mendelsohn, Karas, 1999) have also proposed the idea of a protective effect of oestrogen. If oestrogen lowered the coagulability of females, its mechanism would be to protect against the naturally occurring increased coagulability. Sagripanti and Carpi(Sagripanti, Carpi, 1998) demonstrated that the menopause is accompanied by a significant increase in TAT levels. The mean TAT levels in postmenopausal women are higher than in age matched males. This may explain the cardiovascular protective effect of HRT in postmenopausal women(Brighthouse, 2001). In addition, young women have significantly lower plasma concentrations of antithrombin III compared to males of similar age. The occurrence of the menopause is accompanied by a significant increase in antithrombin III, fibrinogen and Factor VII concentrations(Dolan et al., 1994). Significant sex differences have also been observed in the plasma concentrations of tissue factor pathway inhibitor(Sagripanti, Carpi, 1998).

Further evidence of a sex hormone influence on coagulation is taken from the clinical and experimental studies into the effects of HRT. Several large studies have consistently shown an increased risk of venous thromboembolism in women using HRT(Daly et al., 1996; Hoibraaten et al., 1999; Jick et al., 1996; Perez-Gutthann et al., 1997). HRT affects haematological variables relating to coagulation and fibrinolysis, such that there is an increase in thrombin production, thrombin activity Factor VII and protein C, and a decrease in antithrombin III, protein S and fibrinogen(Koh et

al., 1998; Meade, 1997; Winkler et al., 2000). In addition, vWF, soluble thrombomodulin, and tPA are all significantly reduced after 6 weeks of HRT treatment(Lip et al., 1997). Winkler et al(Winkler et al., 2000) have suggested that the overall haemostatic balance is shifted towards a profibrinolytic response by the commencement of HRT. Other groups, have argued that there is no consistent evidence of a prothrombotic tendency in HRT patients(Douketis et al., 2000) and Koh et al(Koh et al., 1998) have suggested that the procoagulatory response is matched by an equivalent increase in fibrinolysis.

The hepatic expression of genes for several coagulation and fibrinolytic proteins are also known to be regulated through oestrogen receptors(Mendelsohn, Karas, 1999). It appears that the net effect of oestrogens on coagulation depends on the form used, the dose, the method of administration and the duration of therapy.

Indirect effects of sex hormones on haemostasis may be due to changes of the vasomotor tone in vessel walls, to activation of endothelial cells or stimulation of the bone marrow resulting in alterations of blood flow, and activation of coagulation, fibrinolysis and blood rheology(Winkler, 1996). It has been suggested that the postmenopausal increased risk of arterial disease is partly mediated via changes in haemostatic variables as a consequence of oestrogen deficiency(Winkler et al., 2000).

Jern et al(Jern et al., 1991) demonstrated that compared to males, females showed lesser stress induced responses in Factor VII:C, vWF:Ag and tPA, in spite of a similar degree of sympatho-adrenal activation.

Thus, sex steroids may influence haemostatic function via genomic effects on gene expression or via non-genomic effects on the activation of plasma membranes. It is conceivable that both mechanisms are involved in vivo. Steroids may derange the reservoir of factors and inhibitors as well as the assembly of intra and extracellular cofactors thereby inducing or enhancing a latent imbalance of pro/anticoagulatory and pro/antifibrinolytic forces(Winkler, 1996).

This study demonstrates that extreme care must be taken when using TEG for clinical and research purposes. Observed changes must always be referenced to the sex of the subject. It can be seen from Figures 17,18 and 19, that the overall mean value for each of the TEG parameters is an average of the hypercoagulable females and the relatively hypocoagulable males. In assessing the potentially small changes observed in research studies, this must always be taken into account, to ensure that bias and thus incorrect conclusions do not occur. Although the sample size was not large, male and female controls were well matched for age, the exclusion criteria for confounding factors such as drugs were rigorously enforced, and the levels of significance in the data are extremely high.

Conclusion

There are sex differences in TEG variables in a control population. Females tend to be hypercoagulable. Within the range of the control population studied, there was no effect of age on TEG variables.

We question the clinical and research applicability of the sample type directed reference ranges generated with the commercially available TEG software.

Control study 3

The primary aim of this study is to ascertain the reproducibility of TEG over time in a control population.

Method

A total of 20 of the subjects in control study 2, were sampled for a second time 14 days following their first test. This group comprised 10 females mean age 25.9 yrs (SD 6.51), and 10 males mean age 33.9 yrs (SD 10.48).

Statistical analysis

To compare the results in the same subject 14 days apart, a paired sample t-test was performed.

Results

The t-tests comparing the values for R, MA and G showed that there was no statistical difference between the samples taken 14 days apart for R, $t = 0.699$, $df = 19$, $p > 0.05$ (Tables 14 and 17, and Figure 20); MA, $t = -1.137$, $df = 19$, $p > 0.05$ (Tables 14 and 19, and Figure 22); and G, $t = -1.163$, $df = 19$, $p > 0.05$ (Table 14).

The t-test comparing the K time however, showed that there was a difference between the 2 samples, $t = 2.883$, $df = 19$, $p < 0.05$ (Tables 14 and 18, and Figure 21). A similarly significant result was also observed for the angle value, $t = -2.578$, $df = 19$, $p < 0.05$ (Table 14).

These results posed the question was it the machine or the subjects that were not stable over time? Having identified differences related to sex in the control data, paired t-tests were carried out on the male and female data separately (Tables 15 and 16).

These results demonstrated that there was no statistically significant difference for any TEG variable in male controls. However, for females there was still a significant difference for K time, $t = 2.761$, $df = 9$, $p < 0.05$, and for Angle $t = -2.701$, $df = 9$, $p < 0.05$.

	N	Day 0 Mean (SD)	Day 14 Mean (SD)	Mean Difference	t	Significance Value
R	20	529.65 (162.80)	503.5 (158.29)	26.15	0.699	0.493
K	20	187.90 (55.42)	157.80 (49.55)	30.10	2.883	0.010
Angle	20	51.68 (8.14)	55.65 (7.92)	-3.98	-2.578	0.018
MA	20	53.68 (4.81)	55.65 (5.42)	-1.98	-1.137	0.270
G	20	5910.38 (1197.48)	6439.68 (1451.97)	-526.30	-1.163	0.259

Table 14. Summary table of paired t-tests for all TEG variables on samples taken 14 days apart.

Using $p < 0.05$ to determine significance, paired t-tests were carried out on all five TEG parameters to see if the samples taken on day 0 were statistically different from those taken 14 days later.

There was no significant difference in TEG parameters R ($p > 0.05$), MA ($p > 0.05$) and G ($p > 0.05$).

There were significant differences for TEG parameters K ($p < 0.05$) and Angle ($p < 0.05$).

Table 15. Males

	N	Day 0 Mean (SD)	Day 14 Mean (SD)	Mean Difference	t	Significance Value
R	10	587.10 (202.78)	517.30 (168.54)	69.80	1.179	0.268
K	10	212.30 (57.73)	182.70 (50.94)	29.60	1.611	0.142
Angle	10	48.40 (7.87)	51.65 (7.97)	-3.25	-1.238	0.247
MA	10	51.35 (5.47)	53.85 (6.09)	-2.50	-7.78	0.457
G	10	5419.25 (1412.78)	6011.40 (15.9.90)	-592.15	-0.738	0.479

Table 16. Females

	N	Day 0 Mean (SD)	Day 14 Mean (SD)	Mean Difference	T	Significance Value
R	10	472.20 (86.61)	489.70 (155.13)	-17.50	-0.394	0.703
K	10	163.50 (42.77)	132.90 (34.80)	30.60	2.761	0.022
Angle	10	54.95 (7.35)	59.65 (5.76)	-4.70	-2.701	0.024
MA	10	56.00 (2.64)	57.45 (4.21)	-1.45	-0.948	0.368
G	10	6401.50 (703.76)	6867.95 (1327.89)	-466.45	-0.973	0.356

Table 15. Summary table of paired t-tests for males.**Table 16. Summary table of paired t-tests for females.**

Using $p < 0.05$ to determine significance, paired t-tests were carried out on all five TEG parameters for males and females separately to see if the samples taken on day 0 were statistically different from those taken 14 days later.

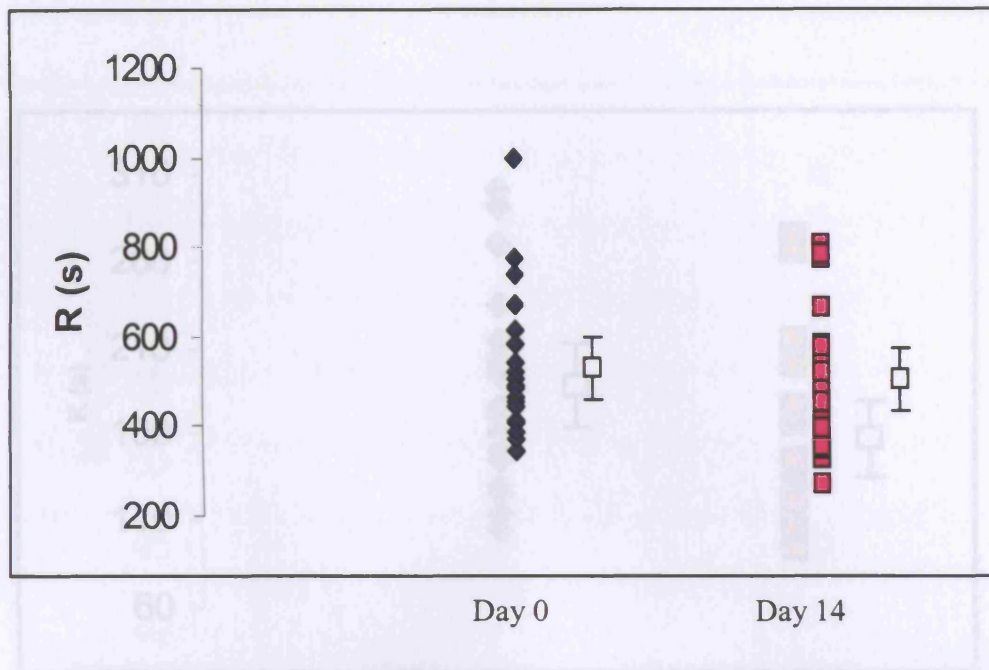
There was no significant difference found for any of the TEG parameters in males. There was no significant difference found for the TEG parameters R ($p > 0.05$), MA ($p > 0.05$) and G ($p > 0.05$) in the females.

However, significant differences for TEG parameters R ($p < 0.05$) and Angle ($p < 0.05$) remained for females.

Using a significance level of $p < 0.05$ you could expect simply by chance, 1 test in 20 to prove significant. A total of 15 t-tests had been performed on this data and 4 had shown significance. The K time and Angle however, are closely related and dependent on each other. Thus, additional statistical analysis was carried out to investigate these findings further.

A regression analysis was performed on the square of the difference between the TEG variables taken on day 0 and on day 14. The data was regressed for age and sex. The rationale behind this was that a positive difference between variables would have been cancelled out by a negative difference, since the test is a comparison of means. By using the square of the difference, the difference is always positive, and always emphasised in the statistical analysis.

Using ANOVA regression analysis, no significant difference was observed in any TEG parameter (R, K, Angle, MA and G) between day 0 and day 14, in male or in female controls (Appendix VI, Tables 1 to 5).



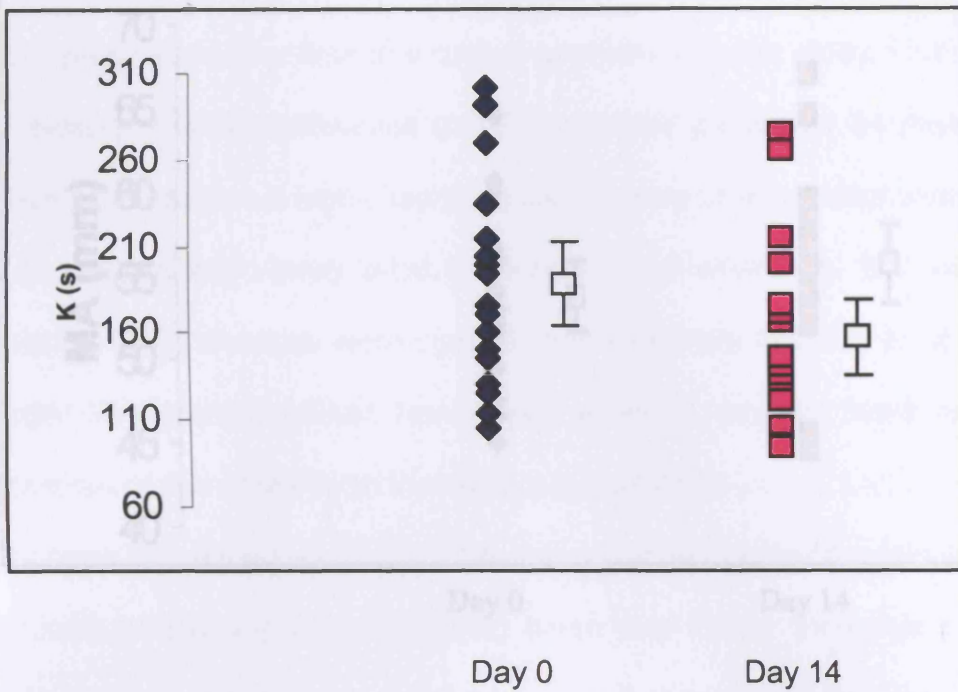
	Mean	Standard Deviation	Confidence Interval
Day 0	529.65	162.80	71.35
Day 14	503.5	158.29	69.37

Figure 20. Scatter diagram of R on samples taken 14 days apart.

There was no significant difference between Day 0 and Day 14 for TEG parameter R ($p > 0.05$).

Table 17. Summary table of R on samples taken 14 days apart.

Table showing the mean, SD and CI on day 0 and day 14.



	Mean	Standard Deviation	Confidence Interval
Day 0	187.9	55.42	24.29
Day 14	157.8	49.55	21.71

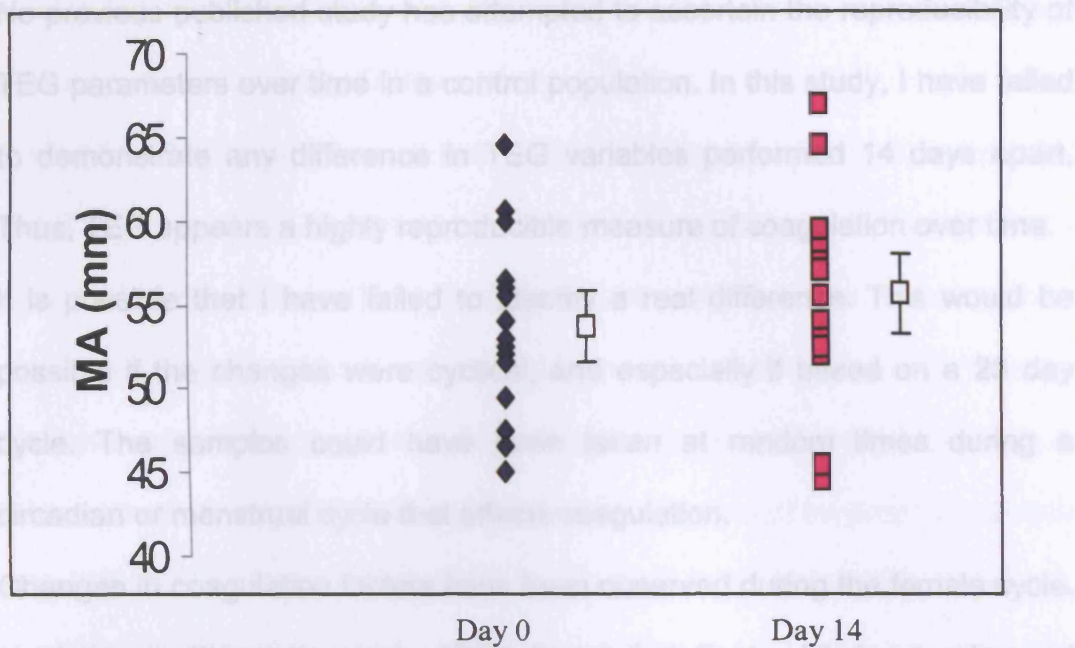
Figure 21. Scatter diagram of K on samples taken 14 days apart.

There was no significant difference between Day 0 and Day 14 for TEG parameter K ($p > 0.05$).

Table 18. Summary table of K on samples taken 14 days apart.

Table showing the mean, SD and CI on day 0 and day 14.

Discussion



	Mean	Standard Deviation	Confidence Interval
Day 0	55.65	7.92	3.47
Day 14	53.68	4.81	2.11

Figure 22. Scatter diagram of MA on samples taken 14 days apart.

There was no significant difference between Day 0 and Day 14 for TEG parameter MA ($p > 0.05$).

Table 19. Summary table of MA on samples taken 14 days apart.

Table showing the mean, SD and CI on day 0 and day 14.

Discussion

No previous published study has attempted to ascertain the reproducibility of TEG parameters over time in a control population. In this study, I have failed to demonstrate any difference in TEG variables performed 14 days apart. Thus, TEG appears a highly reproducible measure of coagulation over time. It is possible that I have failed to identify a real difference. This would be possible if the changes were cyclical, and especially if based on a 28 day cycle. The samples could have been taken at random times during a circadian or menstrual cycle that affects coagulation.

Changes in coagulation factors have been observed during the female cycle. Kapiotis et al(Kapiotis et al., 1998) found that Factor VII levels changed during the menstrual cycle and they argued that this supported the idea that coagulation factors may be up or down regulated by sex hormones.

Jern et al(Jern et al., 1991) have shown that both gender and physiological variations in female sex hormones may modulate the haemostatic response to psychosocial stress. At rest they compared coagulation markers in the follicular and luteal phases of the cycle in 9 women. The platelet count was higher in the follicular phase. The haematocrit and vWF concentration was higher in the luteal phase. The resting levels of factor VII:C, VII:Ag, tPA and PAI were all unchanged at rest. This paper also provided evidence for a different haemostatic stress response between males and females. Wramsby et al observed very little change in the response to activated protein C throughout the female cycle(Wramsby et al., 2001).

Jespersen and Kluff(Jespersen, Kluff, 1986) provided evidence of a cyclical change of tPA inhibition, and showed how this changed following the administration of differing OCPs.

It would be logical to assume that during the female cycle there would be equal time periods of hypo and hypercoagulability, presumably mediated by endogenous hormones. This study was not controlled for the menstrual stage of female controls. By chance you would expect half to have been in the hypocoagulable phase and half to be in the hypercoagulable stage, if these phases existed. Therefore, half the changes would be positive and half negative. Thus, these changes could have cancelled each other out, revealing statistically no change. I have attempted to circumvent this by in addition analysing the square of the difference between the values. This should have amplified any difference present. Regression of this data also failed to reveal any difference in the TEG variables over time.

Control study 2 shows that there does appear to be differences in TEG parameters between males and females. This is presumably under some form of hormonal control or influence. Therefore, it would not be surprising to observe a change in coagulation with the female cycle.

TEG parameters appear to be hypercoagulable in pregnancy(Gorton et al., 2000), suggesting a hormonal association. Oestrogen plasma levels change with different phases of the menstrual cycle. As oestrogen is known to effect the levels of certain clotting factors, it would be logical that this could affect the process of coagulation. Therefore cyclical changes in hormones may mean a cyclical change in the mechanisms of haemostasis.

The effects that sex hormones play on coagulation responses can be ascertained by looking at the changes observed following the introduction of the oral contraceptive pill. Differences in fibrinogen, thrombin-antithrombin (TAT), prothrombin fragment 1 and 2, antithrombin III, protein C, protein S, tissue plasminogen activator, plasminogen and plasminogen activator inhibitor have all been observed after starting combined oral contraceptive preparations(Prasad et al., 1999). Other studies have observed that in women taking the oral contraceptive pill, there is an increase in the activity of the procoagulant Factors VII, X, and fibrinogen occurs, and a fall in anticoagulant antithrombin III(1999a).

Further evidence that there is a hormonal influence on coagulation are the changes in coagulation markers observed in postmenopausal women, which appear to be at least partly reversible with hormone replacement therapy(Sagripanti, Carpi, 1998).

In theory, the male coagulation response should remain fundamentally more stable, as the male is not predisposed to such large hormonal fluctuations.

Conclusion

TEG is a reproducible measure of coagulation over time in male and female controls.

I have failed to identify any cyclical changes in coagulation in male or female controls using TEG.

Experimental studies

Studies in haemostasis following subarachnoid haemorrhage

Literature review

Subarachnoid haemorrhage (SAH) occurs at all ages but strikes primarily in middle age, often with devastating consequences. It is estimated that about 40% of patients admitted to hospital die within 1 month and more than one third of survivors have severe disability(Schievink, 1997). Even in patients admitted to hospital in a good clinical grade (WFNS 1 or 2), approximately one third will have a poor outcome(Roos et al., 2000a). SAH accounts for a quarter of cerebrovascular deaths(Wardlaw, White, 2000). SAH is due to the rupture of an intracranial saccular aneurysm in about 75% of cases, which usually arise from the circle of Willis or a branch artery(Schievink, 1997).

Several studies have indicated that smoking and hypertension are important risk factors for SAH due to aneurysms(Ruigrok et al., 2001). Genetic factors may also have a role as more close relatives of aneurysm patients harbour intracranial aneurysms than the background population(Gaist et al., 2000; Teunissen et al., 1996).

Over the last 20-30 years there has been a remarkable improvement in the results reported after surgery for ruptured intracranial aneurysms. Operative mortality has fallen from over 20% to less than 5%(Hop et al., 1997; Maurice, Kitchen, 1994). However, despite considerable advances in diagnostic, surgical and anaesthetic techniques, and perioperative management, the outcome for patients with SAH remains poor, with overall mortality rates of at least 25% and significant morbidity among approximately 50% of survivors. When even more detailed outcome assessments of SAH patients have been performed in structured studies, it is clear that very few patients return to their level of premorbid function following the ictus(Hop et al., 1998). Well

known clinical predictors of mortality at 1, 6 and 12 months following SAH include, the level of consciousness on admission, age (>65 years), presence of comorbid disease, such as hypertension, and neurological signs on admission(Arboix, Marti-Vilalta, 1999; Germanson et al., 1998; van Gijn, 1992; Vermeulen, 1996).

Population based incidence rates for SAH vary from 6 –16 per 100000, with the highest rates reported in Finland and Japan(Broderick et al., 1993; Kiyohara et al., 1989; Linn et al., 1996; Sarti et al., 1991). The most accurate estimate of the incidence in the UK is 9.7 per 100000(Pobereskin, 2001). Unlike other types of stroke, the incidence of SAH has not declined over time. The incidence of SAH increases with age (mean age of approximately 50 years), and is higher in women than in men(Ingall et al., 1989).

The evolution of treatment protocols for patients with SAH has been influenced by large, multicentre prospective, randomised, cohort analyses and, more recently, by multicentre prospective, randomised trials. Nevertheless, several accepted treatment modalities have not been substantiated by rigorous clinical scientific assessment. In many cases specific treatments for SAH are not amenable to testing by randomised, prospective trials because of practical or ethical considerations.

Vasospasm, as well as rebleeding poses a serious problem of prognostic importance in patients with aneurysmal SAH. Although there are number of effective methods for improving symptomatic vasospasm (hypervolaemic therapy, percutaneous transluminal angioplasty, intraarterial papaverine infusion), due to their serious side effects, these methods are not completely safe. Therefore, a means of predicting whether delayed ischaemic

neurological deficits (DINDs) were likely to occur, would allow clinicians to identify and intensively treat only those patients at greatest risk of DIND.

One major unresolved controversy in the management of ruptured intracranial aneurysms is the timing of surgery. Should this be performed early or late? Very early surgery in good condition patients is becoming widespread. It is argued that this prevents not only rebleeding, but may also help prevent ischaemia by enabling the basal cisterns to be cleared of blood, thereby reducing the incidence of vasospasm, and permitting postoperative hypertensive treatment to aggressively manage delayed cerebral ischaemia(Yoshimoto et al., 1999). On the other hand, it is likely that early surgery is associated with a higher mortality and morbidity than surgery delayed until after the period of cerebral instability following rupture of an intracranial aneurysm(Kassell et al., 1990a; Maurice, Kitchen, 1994; Maurice-Williams, 1987).

Haemostatic indices are relevant to this debate. Thus, if there are two phases of haemostasis following a SAH; an initial period of hypocoagulability followed by a second phase of hypercoagulability. In SAH if rebleeding is related to hypocoagulability, which can be measured by bench techniques, then perhaps pharmacological manipulation of coagulation could be focussed on the administration of antifibrinolytic agents to those who have not entered the period of fibrinolytic shutdown. A reduction of the early rebleeding risk would allow for delayed surgery and significantly impact on the outcome of haemorrhage. Conversely, if the period of hypercoagulability can be correlated to the period when patients are at greatest risk of DIND, then early surgery followed by anticoagulation may impact on the outcome.

Haemostatic changes and SAH

Although spontaneous SAH has been extensively studied from various standpoints, few reports have assessed changes in the haemostatic systems occurring in association with SAH.

Fujii et al (Fujii et al., 1997) compared the serial changes in haemostatic systems after the clipping of ruptured aneurysms, and compared this with those seen after the clipping of unruptured aneurysms for a period of 30 days.

Blood cells. They observed no change in the white cell count, haematocrit and platelet count between the 2 groups. The white cell count gradually decreased to within its normal range within 1 month of SAH. The change in platelet count was biphasic. The initial decrease possibly reflected haemodilution and consumption after surgery, whereas the subsequent elevation was thought to represent the physiological response to injury.

Blood coagulation. The change in fibrinogen levels was monophasic and very similar in the 2 groups. The plasma level reached a peak on day 3. The level of fibrinogen, a representative acute phase reactant, never decreased significantly following SAH.

The plasma level of thrombin-antithrombin complex, although highest on admission and gradually decreasing thereafter, never returned to within its normal range. The complex levels on day 3 and 6 were higher in the SAH group. Hence, patients with SAH appeared to be in a hypercoagulable state within the 1st month following SAH which was not attributable to the surgery itself.

Fibrinolysis. The change in the plasma level of plasmin-antiplasmin complex was biphasic. Although the complex reached its highest level on day 6 and gradually decreased, it did not return to its normal level. Thus, patients with SAH also appear to be in a state of hyperfibrinolysis during the initial month, and especially in the 2nd week following the ictus.

The change in D-dimer was monophasic, with the plasma level reaching a peak after day 3 and gradually decreasing thereafter. However, the plasma levels of D-dimer were far higher than in the patients who underwent clipping of ruptured aneurysms. Because D-dimer is a fragment of cross-linked fibrin digested by plasmin, the apparent elevation of D-dimer may be attributable to the entry of dissolved subarachnoid clot into the blood circulation with CSF.

In this study, the authors compared the results in patients with, and without a DIND, to see if any of the above factors could be used to predict the development of ischaemia. There was no significant difference in any of the haemostatic parameters on day 0. However, on day 3, that is, before the appearance of DINDs, the plasma levels of fibrinogen and D-dimer were significantly higher in those patients who subsequently developed DINDs than in those who did not. The levels of fibrinogen and thrombin-antithrombin complex on day 6, and the level of D-dimer on day 14 in the patients with DINDs were also significantly higher.

Nina et al (Nina et al., 2001) observed a less clear association between coagulation and fibrinolytic makers following SAH. They studied 76 patients, however, 52 of these were treated with antifibrinolytics and dexamethasone which may have altered the levels of factors measured. All had blood samples were drawn within 24 hours of the SAH.

In this study PT, APTT and fibrinogen were within normal limits. TAT levels in the plasma paralleled the severity of the neurological state, but did not reach statistical significance. TAT levels appeared to be associated with clinical outcome because 16 patients out of 27 (59%) with unfavourable outcomes displayed TAT levels >20 ng/ml, as compared with 10 patients out of 38 (26%) with favourable outcomes.

Plasma d-dimer was invariably raised and unrelated to clinical grade at admission. Nevertheless, very high values of d-dimer (>1000 mcg/ml) were found in 16 out of 22 patients (73%) with unfavourable outcomes, but in only 9 patients out of 38 (24%) with favourable ones. D-dimer showed a weak association with DIND, whereas a stronger association was observed with DIND severity. In this study 14 patients out of 76 (18.4%) developed DIND, which was responsible for poor outcome in 10. In 9 of these 10, d-dimer levels rose above 1000 mcg/ml.

Itoyama and colleagues(Itoyama et al., 1994) examined the relationship of thrombin-antithrombin (TAT) and plasmin- α_2 -plasmin inhibitor complex (PIC) in 51 patients with non traumatic SAH. They investigated the correlation of these levels with the severity of the disease, the incidence of arterial spasm, and the outcome.

The TAT and PIC on admission both showed a positive correlation with Hunt and Hess grade. There was a less significant correlation between TAT and PIC on admission and Fisher grade. This association was lost between TAT and Fisher grade 4.

There was no association between either TAT, or PIC on admission, and the occurrence of arterial vasospasm. However, a lower TAT or PIC on admission correlated with improved neurological outcome on discharge.

The authors also evaluated the chronological changes in TAT and PIC levels in 44 of the patients. The mean TAT level on admission was 27.3 +/- 20.5 ng/ml, and 1 week later 13.0 +/- 10.9 ng/ml. The mean PIC value was 2.50 +/- 3.11 µg/ml at the time of admission, and 1.09 +/- 0.89 µg/ml 1 week later. These differences were significant.

Fujii et al (Fujii et al., 1995) examined the changes in haemostatic systems within 24 hours following spontaneous SAH, and their relationship to neurological grade, blood load on admission CT and clinical outcome. The haematological factors examined were prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, antithrombin, protein C, thrombin-antithrombin complex (TAT), plasminogen, α_2 -antiplasmin, d-dimer, plasmin-antiplasmin complex (PAP), platelet count, platelet sensitivity and white blood cell count (WCC).

Relationship with neurological grade. The levels of WCC, TAT, d-dimer, and PAP significantly increased with neurological severity, and almost all were elevated beyond the normal range. All other parameters were not statistically different. In addition, the poor outcome rate significantly increased with the neurological severity.

Relationship with blood load. The levels of WCC, TAT, d-dimer, and PAP significantly increased with the amount of subarachnoid clot. There was a significant increase in poor outcome rate as the amount of clot increased.

Relationship with clinical outcome. The levels of TAT, d-dimer and PAP significantly increased with the severity of clinical outcome. The other parameters, including WCC, did not change in any significant association with the severity of clinical outcome.

Relationship of TAT complex levels and clinical outcome. There was a significant difference in the TAT complex levels between patients with good and poor outcomes. None of the 61 patients whose TAT was ≤ 50 ng/ml had a poor outcome. Furthermore, none of the 18 patients whose complex level was > 250 ng/ml achieved a good outcome. The poor outcome rate was 55.3% in 47 patients whose level was >50 and ≤ 250 ng/ml.

This study also had a cohort of ICH and cerebral infarction patients whose coagulation system was activated far less than following SAH.

The WCC is an acute phase reactant, which increases in response to various stimuli (infection), therefore its increase does not indicate a specific physiological response. On the other hand elevations of TAT, d-dimer and PAP, demonstrate the activation of the blood coagulation system, the dissolution of blood clots, and the activation of the fibrinolytic system respectively. Thus, this study demonstrates the activation of the coagulation and fibrinolytic systems following SAH and that activation to a greater extent appeared to be related to the severity of neurological grade, CT blood load and clinical outcome. The authors concluded that the levels of plasma coagulation markers, especially TAT, may reflect the severity of the clinical state of the patients with SAH, and may contribute to predicting the clinical outcome in these patients.

In head trauma, DIC is thought to be due to the release of large quantities of thromboplastin into the circulation from the damaged brain inducing a hypercoagulable and hyperfibrinolytic state (Itoyama et al., 1994). In the acute stage of SAH, various events, such as intimal damage to the cerebral vessels, the release of thromboplastin from the damaged brain, the elevation of biogenic amines (epinephrine, norepinephrine and serotonin) in the serum, which occurs in response to stress and stasis in the bloodstream, and/or systemic dehydration, predispose to platelet hyperactivity and/or hypercoagulation disorders.

Perhaps the formation of microthrombi within the small cerebral vessels, associated with vasospasm and hypercoagulability, could predispose to DIND and ischaemic symptoms in these patients.

Antifibrinolytic, antiplatelet or anticoagulant medication may be indicated to improve the cerebral microcirculation.

Haemostatic changes and spontaneous intracerebral haemorrhage

While impaired haemostasis is a risk factor for ICH only one report has looked at the immediate effect of ICH on coagulation and fibrinolysis in detail. Fujii and colleagues (Fujii et al., 2001) examined these markers in 358 patients within 6 hours of their ICH. Underlying vascular abnormalities were excluded by angiography.

In ICH patients showing neither, intraventricular extension or SAH, the mean values of the haematological parameters examined (TAT, PAP and d-dimer), with the exception of WCC were not significantly higher than in normal controls. There was no difference in haemoglobin concentration, platelet count, platelet sensitivity, fibrinogen, PT or APTT.

The levels of the WCC, TAT, PAP, and d-dimer were significantly higher in patients with intraventricular extension, or SAH, in addition to the ICH. The other haematological parameters were not changed. Multiple logistic regression analysis revealed that, TAT and WCC were significantly and independently associated with intraventricular and SAH. The WCC, TAT, PAP and d-dimer levels, significantly increased with the amount of intraventricular and SAH. In patients with no intraventricular or SAH, the volume of the ICH was not associated with any of the factors measured.

Thus, in ICH patients, intraventricular or SAH appears more responsible for the systemic activation of haemostatic systems than the intracerebral (intraparenchymal) haemorrhage.

Haemostatic changes and head injury

Coagulation abnormalities consistent with a state of disseminated intravascular coagulation (DIC) have been shown to occur as a result of acute head injury (Kumura et al., 1987; Miner et al., 1982; Olson et al., 1989; Pondaag.W., 1979; van der Sande et al., 1981). Moreover, these studies have demonstrated a correlation between the severity of head injury and the degree of DIC. Several investigators (Kaufman et al., 1980; Kumura et al., 1987; Olson et al., 1989; Ueda et al., 1985) have shown that the degree of brain tissue destruction in head injury, appears to correlate positively with the level of fibrin degradation products (FDP) present in the plasma. Preston et al (Preston et al., 1974), and Stein et al (Stein et al., 1992), implicated microthrombosis as a consequence of disseminated intravascular coagulation (DIC), in the ischaemic damage following head injury.

Selladurai et al (Selladurai et al., 1997) investigated the prognostic value of coagulation abnormalities in 204 patients with acute closed head injury. They measured PT, APTT, thrombin clotting time, fibrinogen, platelet count, and FDP count within 48 hours of head injury, and calculated a DIC score from this, by grading the values 0-3. The DIC score correlated inversely with the GCS. When the GCS was fixed, both DIC score and FDP score were independent predictors of poor outcome. The predictive value of haemostatic parameters were more striking within specific GCS subsets. In GCS 13-15 patients (n=43), all 11 patients who had a poor outcome had moderate to severe FDP scores. In the GCS 6-12 subset (n=142), out of 47 patients who had significantly abnormal APTT scores only 2 had good outcome. Of the 35 patients who did not have significant abnormal DIC scores, only 2 had poor outcome. The authors suggested that following head injury the following mechanisms might contribute to a state of DIC:

The release of thromboplastins following parenchymal injury, and activation of the extrinsic coagulation cascade. Endothelial damage, with platelet activation and activation of the intrinsic coagulation cascade. Intravascular thrombosis resulting in depletion of platelets and coagulation factors, and finally FDPs acting as anticoagulants and platelet inhibitors.

The authors concluded that, PT, APTT and thrombin clotting time, were insensitive assessments of the state of DIC, as they are not abnormal until a significant reduction in the number of clotting factors occurs. They however concluded that, abnormal haemostatic parameters may enhance prognostic ability in subsets of patients with acute head injury, defined by clinical or CT predictors.

Coagulation mechanisms specific to brain injury

The mechanisms favouring hypercoagulability may differ following different forms of trauma. In addition, it appears likely that post traumatic hypercoagulability is a systemic phenomenon(Dahl, 1999).

Thromboplastin. Although the brain contains the highest concentration of thromboplastin in the human body(Bjorklid et al., 1977; Bjorklid, Storm, 1977), and it is speculated that trauma, infarction and surgery to the brain results in the release of tissue thromboplastin, and activation of the coagulation cascade, there is no direct evidence to support this(Hamilton et al., 1994).

Tissue Factor. Tissue factor (TF) is the primary cellular initiator of blood coagulation and the most potent trigger of blood coagulation known today. The adventitia surrounding the major blood vessels, the brain, and the bone marrow are the structures with high levels of TF(Dahl, 1999). The concentration of TF is reported to be greater in the cortical grey matter than in white matter(del-Zoppo et al., 1992). It is reported to be associated with non capillary microvessels in the cerebral cortex and with the adventitia, as seen in superficial cerebral vessels(Dahl, 1999; Fujii et al., 2001). Thus, the entry of blood into the subarachnoid space may cause a considerable amount of TF release into the systemic circulation through injury to superficial brain tissues, including superficial cerebral arteries, resulting in systemic haemostatic activation.

Dissolved clots in the subarachnoid space enter the blood circulation with CSF to activate the haemostatic systems. Rapidly increasing ICP or severe meningeal stimulation may induce systemic activation of haemostatic

systems through unknown neurogenic and/or humoral mechanisms. However, as the volume of ICH is not related to the amount of haemostatic activation, it appears unlikely to be due to a rise in ICP(Fujii et al., 2001).

Cerebral ischaemic stroke and haemorheology

A number of studies have implicated the haemostatic system in the pathogenesis of stroke, proposing an excess of coagulation factors, fibrinolytic inhibitors or both(Carter et al., 1998; Catto, Grant, 1995; Kain et al., 2001a; Kain et al., 2001b).

It is well known that patients with polycythaemia are prone to strokes and transient ischaemic attacks (TIA's). Thomas et al(Thomas et al., 1977) found that cerebral blood flow in 38 patients with a haematocrit of 0.47-0.53 was significantly lower than in 43 patients with a lower haematocrit in the range 0.36-0.46. They then performed venesection in the higher haematocrit group. Blood flow increased in every case. Lowering the mean haematocrit from 0.493 to 0.426 raised the mean cerebral blood flow by 50% from 41.4 to 62.1 ml/100g/min. The venesected patients were also symptomatically better, including 5 patients with carotid territory TIA's and 4 patients with vertebrobasilar TIA's.

Ischaemic stroke as well as developing in the course of many haematological conditions, may also be its first clinical manifestation. Arboix and Besses(Arboix, Besses, 1997) demonstrated ischaemic stroke to be the initial presentation in cases of essential thrombocythaemia, polycythaemia vera, thrombotic thrombocytopenic purpura, IgA lambda myeloma, acute lymphoblastic leukaemia, Waldenstrom's macroglobulinaemia, chronic granulocytic leukaemia, protein C deficiency and IgG lambda myeloma. In

addition, cases of ischaemia related to antithrombin, protein C and S deficiency are well described. The role of dysfunction in coagulation and fibrinolysis, platelet hyperactivity, erythrocyte abnormalities, myeloproliferative, or hyperviscosity states underlying many diverse haematological disorders in the genesis of stroke is as yet unclear and probably multifactorial.

Polycythaemia vera and the forms of secondary polycythaemia may cause cerebral vascular thrombotic events because of increased red cell mass and blood hyperviscosity. The hyperviscosity syndrome has also been described in a variety of haematological disorders such as paraproteinaemias, in which the presence of monoclonal proteins in the microcirculatory bed accounts for cerebral vascular occlusive episodes.

Faber et al (Faber et al., 1996) identified enhanced red blood cell aggregation in a series of young stroke patients which was not related to abnormal concentrations of fibrinogen, immunoglobulins, or abnormal haematocrit values. They suggested that this was the possible stroke mechanism through hyperviscosity.

Other authors have demonstrated platelet activation in both the acute and convalescent stage of thrombotic stroke (Meiklejohn et al., 2001). They demonstrated increased expression of P-selectin and fibrinogen binding. Others have shown enhanced fibrinogen, vWF and plasminogen activator inhibitor concentrations in the acute and recovery phases of ischaemic stroke (Catto, Grant, 1995).

In summary, haemostasis and stroke have been relatively understudied, particularly in relation to stroke classification, predicting risk of disease, and clinical outcome, with the results obscured by the acute phase reaction.

In acute ischaemic stroke the notion of 'time is brain' has evolved in the acute management. Irreversible ischaemic brain damage evolves over hours and reperfusion can rescue tissue that is functionally inactive, but still viable (the ischaemic penumbra)(Muir, 2001). Several haematorheological treatments have evolved as a result of large randomised trials.

Thrombolysis. Thrombolytic drug treatment for acute ischaemic stroke is now well established in North America. Evidence for this arose from large randomised trials(Hacke et al., 1998; Steiner et al., 1998) of which the NINDS rt-PA was pivotal(The National Institute of Neurological disorders and Stroke rt-PA Study Stroke Group, 1995). This showed a highly significant increase in the proportion of patients making a full neurological recovery from stroke (12% absolute risk reduction, equating to 1 extra patient fully recovered for every 8 treated), with no increase in mortality.

Antiplatelet drugs. Combined data from 3 trials(Chen et al., 2000) involving over 40000 patients indicates that initiation of aspirin 150-300mg within 48 hours of stroke:

Prevents early recurrent ischaemic stroke (absolute risk reduction 0.7%)

Reduces death and disability at 3-6 months (1.3%)

Increases the proportion of patients making a full neurological recovery (1.1%)

At the expense of a small increase in intracranial (0.2%) and extracranial (0.4%) haemorrhage risk

Interestingly, there was no heterogeneity of these effects across any subgroup including intracerebral haemorrhage patients randomised before CT.

Ancrod. Treatment within 3 hours with this snake venom derivative that reduces serum fibrinogen concentrations, significantly increased the proportion of patients making full neurological recovery in a randomised, controlled trial(Sherman et al., 2000).

Heparin. No significant reduction in death or disability after stroke has been found in any trial of heparin, either in its unfractionated or low molecular weight preparations commenced within 24-48 hours after stroke onset, even in subgroups of patients with atrial fibrillation(Bath et al., 2000).

Antifibrinolytic drugs and SAH

Patients who survive aneurysmal SAH have a high morbidity and mortality in the following weeks due mainly to the complications of rebleeding and cerebral ischaemia. Because rebleeding has been attributed to fibrinolysis of the blood clot around the fundus of the aneurysm(Fodstad et al., 1978) there is logic in using antifibrinolytic drugs (epsilon aminocaproic acid, tranexamic acid) in the prevention of rebleeding. Plasmin may be carried to the clot by blood circulating in the aneurysm or by the CSF and leptomeninges surrounding it(Fodstad et al., 1978).

Adams et al(Adams et al., 1981) reviewed the antifibrinolytic experience from three studies (two randomised studies and one prospective phase IV study), which consistently showed a significant reduction in rebleeding among treated patients compared with control subjects. However, nearly one third of these patients were worse at 14 days compared with the time of admission.

Ramirez-Lassepas(Ramirez, 1981) reviewed 25 previous published studies on the use of antifibrinolytics in SAH and was unable to form any firm conclusions or recommendations. In 1984, a multicentre, randomised, double blind, placebo controlled study using tranexamic acid showed that rebleeding was reduced by more than 60% in the treatment group, but an increased rate of cerebral infarction in these patients offset any improvement in overall outcome(Vermeulen et al., 1984). A nonrandomised, control study demonstrated similar findings; a 40% reduction in rebleeding in patients receiving antifibrinolytic therapy was offset by a 43% increase in focal ischaemic deficits(Kassell et al., 1984).

A more recent prospective, double-blind, placebo controlled study(Roos, 2000), randomised 462 patients and compared antifibrinolytics combined with maximal anti-ischaemic therapy. Once again this study confirmed that of others in showing a statistically significant reduction in rebleeding in treated patients, but no overall improvement in patient outcome.

Possible explanations for the increased rate of ischaemic neurological deficits in patients receiving antifibrinolytic therapy include the following. These agents do not increase the severity of vasospasm but may aggravate the intravascular sludging, platelet fibrin emboli, and microthrombosis that accompany the arterial narrowing. Another possibility is that antifibrinolytic agents do increase the severity of vasospasm. The severity of vasospasm is related to the amount and duration of blood in the basal cisterns, and it has been shown that tranexamic acid prolongs the clearance of blood from the subarachnoid space(Fodstad et al., 1981; Kassell et al., 1984).

To determine if a shorter course (4 days) of antifibrinolytic treatment, before the expected onset of ischaemic complications might reduce the rate of rebleeding and yet avoid ischaemic complications, Wijdicks et al studied 119 patients prospectively(Wijdicks et al., 1989). However, at 3 months the outcome was no different to patients treated with long term tranexamic acid. In addition the shorter duration of tranexamic acid failed to prevent rebleeding significantly, as most second haemorrhages occurred after stopping the antifibrinolytic.

The Cochrane review study group examined all randomised, unconfounded, controlled trials in which, after concealed allocation, antifibrinolytic drugs were compared in SAH, in an intention to treat analysis, with control treatment (open studies) or placebo (blind studies)(Roos et al., 1998; Roos et al., 2000b). Eight trials met the predefined inclusion criteria. These included 937 patients of whom 476 were randomised to receive antifibrinolytic drugs; 364 received placebo treatment and 97 patients received open control treatment. This analysis confirmed earlier investigations in showing that antifibrinolytic drugs showed no effect on outcome or death from all causes. It confirmed an overall 45% reduction in rebleeding with their use and no effect on the incidence of hydrocephalus.

Timing of aneurysm surgery

Without operative intervention or antifibrinolytic treatment about 30% of SAH patients have a rebleed within 1 month of the initial ictus(Locksley, 1966). Only 20% of patients who rebleed survive at 3 months and most have severe brain damage(Hijdra et al., 1987).

The international study on the timing of aneurysm surgery(Kassell et al., 1990a; Kassell et al., 1990b) analysed management comparison in 3521 patients, of whom 83% underwent surgical repair of the ruptured aneurysm. Timing of surgery after SAH was significantly related to the incidence of preoperative rebleeding (0 to 3 days; 5.7%, 4 to 6 days; 9.4%, 7 to 10 days; 12.7%, 11 to 14 days; 13.9%, 15 to 32 days; 21.5%). Postoperative bleeding did not differ among time intervals (1.6% overall). However, there was no significant difference in overall outcome if surgery was performed early (0-3 days) or late (11-14 days). Outcome was worse if surgery was performed in the 7-10 day post-bleed period. Surgical results were better for patients operated on after 10 days. Overall, early surgery was neither more hazardous nor beneficial than delayed surgery. The authors concluded that the postoperative risk following early surgery is equivalent to the risk of rebleeding and vasospasm in patients waiting for delayed surgery.

Even in modern units aiming at early aneurysm surgery this is achieved in only 55% of patients within 3 days(Roos et al., 1997). In this study, rebleeding was still the most common cause of morbidity and mortality in SAH patients. However, the most commonly occurring complication was cerebral ischaemia(Roos et al., 2000a).

Cerebral vasospasm following SAH

Cerebral vasospasm remains a devastating medical complication affecting 30-70% of survivors of aneurysmal SAH(AI Yamany, Wallace, 1999). Cerebral vasospasm is the delayed narrowing of large capacitance arteries at the base of the brain after SAH, often associated with radiographic or cerebral blood flow evidence of diminished perfusion in the distal territory of

the affected artery. Angiographic vasospasm has a typical temporal course, with onset 3 to 5 days after the haemorrhage, maximal narrowing at 5 to 14 days, and gradual resolution over 2 to 4 weeks(Heros et al., 1983). In about one half of cases, vasospasm is manifested by the occurrence of a delayed ischaemic neurological deficit (DIND), which may resolve or progress to cerebral infarction. In contemporary series, 15% to 20% of such patients suffer stroke or die from vasospasm despite maximal therapy(Haley et al., 1992; Longstreth et al., 1993). The DIND associated with symptomatic vasospasm usually appears shortly after the onset of angiographic vasospasm with the acute or subacute development of focal or generalised symptoms or signs(Heros et al., 1983; Kassell et al., 1985). Progression to cerebral infarction occurs in approximately 50% of symptomatic cases; recovery without deficit in the remaining individuals may occur despite the persistence of angiographic vasospasm(Heros et al., 1983).

In 1987, the cooperative aneurysm study reported an incidence of angiographic vasospasm of more than 50%, with symptomatic vasospasm in 32% of patients(Adams et al., 1987; Awad et al., 1987). In a comprehensive review, Dorsch(Dorsch, 1995) reported that despite treatment with nimodipine, only 51% of patients with a DIND due to vasospasm made a good neurological recovery.

If vasospasm decreases cerebral blood flow below 35ml/100g/min, brain tissue pH will decrease, and severe acidosis is associated with neuronal injury.

While areas of cerebral infarction are often related to the major arteries and changes suggestive of infarction can often be seen on CT imaging following

SAH, the relationship is not clear. Patients may deteriorate in the presence of a relatively normal CT scan and yet patients with obvious ischaemic areas on imaging will appear clinically unaffected by such an event. Neil-Dwyer et al (Neil et al., 1994) studied the cortical and hypothalamic changes in 53 SAH patients who had died. Cortical ischaemic changes were present diffusely in 78% of SAH patients studied. There was no clear relationship between either the presence of large vessel vasospasm or the amount of subarachnoid blood, and the observed cerebral ischaemia. Hypothalamic ischaemia was observed in 50% of the post mortems studied. Ischaemic changes were more common in patients with a fluctuating blood pressure, however the mechanisms producing the ischaemia were not postulated by the authors. However, they did localise the anatomical area to the cerebral microcirculation. They suggested that the extensive distribution of lesions might help explain some of the neurological sequelae following SAH, especially with regard to psychosocial and cognitive problems. In particular the lack of drive, loss of concentration, memory deficits and lethargy frequently reported.

In a recent study analysing the prognostic factors associated with the occurrence of cerebral vasospasm after SAH, in a singular population of 244 patients treated either by endovascular coils or conventional surgical treatment (Charpentier et al., 1999). An age ≤ 50 years, WFNS clinical grades ≤ 2 , and hyperglycaemia were independently and significantly associated with the occurrence of cerebral vasospasm. In contrast, no relationship was found with the type of treatment. The 6 month risk of neurological sequelae

was quadrupled when cerebral vasospasm occurred after SAH, and strongly increased with treatment complications and secondary brain insults.

Interestingly, MR and perfusion studies have shown cerebral oedema in the areas of cortex corresponding to vasospastic cerebral vessels, with the relative blood volume significantly higher in the oedematous regions of poor, compared to good grade patients(Rowe et al., 1998). This suggests a compensatory dilatation of the capillary bed in response to proximal vasospasm. However, the microcirculatory changes, especially whether vessels dilate or not during vasospasm, are controversial and still uncertain(Ohkuma et al., 2000).

Ohkuma and colleagues(Ohkuma et al., 2000) showed that severe angiographic vasospasm was statistically correlated with reduced regional cerebral blood flow and prolonged peripheral cerebral circulation time. They suggested that the prolonged peripheral cerebral circulation time resulted from microcirculatory changes producing cerebral ischaemia during vasospasm. These results suggest that impaired autoregulatory vasodilatation or decreased luminal calibre in the intraparenchymal vessels may play a part in the ischaemia induced by cerebral vasospasm. These authors suggested that, the therapy for cerebral vasospasm should be reconsidered by taking into account the microcirculatory changes. Induced hypertension and hypervolaemia might improve regional cerebral blood flow by affecting small vessels with increased resistance. The effectiveness of calcium channel blockers, despite the absence of apparent effect on angiographic vasospasm may partially depend on dilating the contractile arterioles. The intraarterial injection of papaverine may have an influence on

intraparenchymal small vessels in addition to large extraparenchymal arteries.

Mechanisms of cerebral vasospasm

Sustained narrowing of large cerebral vessels remains a major complication after aneurysmal SAH. Despite intensive research, its pathogenesis is still a matter of debate, and adequate pharmacotherapy has been elusive. The absence of adequate therapy continues to motivate preclinical and clinical studies. Other complicating findings are that, vasospasm is not necessarily associated with SAH following traumatic head injury, that the timescale for maximal vasospasm seems different in traumatic SAH, and it appears to rarely produce a clinical deterioration in head injured patients(Zubkov et al., 2000).

While cerebral vasospasm is more common following aneurysmal rather than traumatic SAH, there have been more recent reports of it occurring rarely after cranial base tumour resection(Bejjani et al., 1999). A large tumour size, the need for preoperative embolisation indicating increased tumour vascularity, prolonged operative time reflecting an increased intraoperative blood loss, preoperative vessel encasement and narrowing, correlated with a higher incidence of postoperative vasospasm. This angiographically demonstrated vasospasm responded well to hypertensive, hypervolaemic and haemodilutional therapy and early use of transluminal angioplasty.

Evidence has accumulated that blood products and their oxygen free radical lipid peroxidation, may play an important role in the both arterial vasospasm and ischaemic cell death. Intimal proliferation, inflammation and fibrosis of

the arterial wall leading to structural narrowing have also been blamed (Al Yamany, Wallace, 1999).

Another possible explanation of the occurrence of vasospasm may be the role of surgical mechanical manipulation (Miyaoka et al., 1993; Murayama et al., 1997). In addition, rather than the presence of erythrocytes, both clinical (Brouwers et al., 1992) and experimental (Shishido et al., 1994) studies have argued in favour of vascular and systemic reactions to the rupture of an artery.

Glyceryl nonivamide (GLNVA) is a potent vasorelaxant. It mediates its effect by opening vascular potassium channels via a calcitonin gene related peptide pathway. Lin and colleagues (Lin et al., 2001) demonstrated in a rabbit model of SAH, that intrathecal GLNVA had a dose dependent protective effect on vascular spasm in the basilar artery. Another potassium channel opener cromakalim, has been shown to topically relax vessels undergoing spasm after SAH in the same model (Zuccarello et al., 1996). Because of toxic side effects, neither of these compounds has been tested in man.

Delayed cerebral vasospasm remains an unpredictable and inadequately treated complication of aneurysmal SAH. Despite intensive research, its pathogenesis is still a matter of debate, and adequate pharmacotherapy has been elusive.

Suzuki et al (Suzuki et al., 2000) noted that an increase in the endothelin 1 concentration from cisternal CSF appeared to precede the onset of cerebral vasospasm. Endothelin 1 (ET-1) is a potent vasoactive 21 amino acid peptide that is a member of a large family of endothelins (1,2 and 3). The

three members of the family are produced in a variety of tissues and may modulate hormone production, cell proliferation and vasomotor tone. Endothelin 1 has been identified in animal models as a powerful vasoconstrictor. Endothelin 1 has been implicated in a number of pathological processes characterised by vasoconstriction, and also in angiogenesis(Dawas et al., 1999).

However, Nishizawa and colleagues(Nishizawa et al., 2000) recently demonstrated, using a two-haemorrhage canine model, that although ET-1 initiated the development of vasospasm through a protein kinase C activation in vascular smooth muscle cells, its value returned to normal within 7 days and thus, ET-1 did not appear to contribute to the maintenance of prolonged vasospasm.

Fassbender et al(Fassbender et al., 2001) studied the effects of the inflammatory cytokines IL-1 β , IL-6 and TNF- α , in the blood and CSF following aneurysmal SAH and the mean CBF measured by transcranial doppler. In 20 patients they measured these values longitudinally over time at 1, 2, 3, 5, 7, 9 and 11 days following SAH, and compared the values with 20 control subjects. All inflammatory markers were dramatically increased following SAH. The CSF concentration of IL-6 was increased 10,000 fold. Longitudinal analysis showed a subacute response for cytokines, peaking between days 5 and 9 and declining thereafter. In SAH, concentrations of IL-1 β and IL-6 were significantly lower in plasma than CSF, perhaps indicating the intrathecal origin of these mediators. The mean CBF increased during the study period, peaking between days 7 and 11 when values reached a plateau. Interestingly, the changes in cerebral blood flow paralleled those of

the CSF concentrations of inflammatory mediators. The response in the CSF slightly preceded those of the haemodynamic abnormalities, peaking between days 5 and 9. Those patients with higher CBFs (≥ 210 cm/s) were associated with significantly increased CSF concentrations of IL-1 β , IL-6 and TNF- α compared to those patients with lower CBF (≤ 210 cm/s).

This study showed that release of IL-1 β , IL-6 and TNF- α in the subarachnoid space of patients with SAH, is associated with the development of increased CBF in the basal cerebral arteries, suggesting a role of excessive subarachnoid inflammatory host response in the haemodynamic complications of SAH.

The pathophysiological importance of compartmentalised cytokine release following SAH may also explain the severe vasospasm in experimental subarachnoid inflammation (Peterson et al., 1990) or in bacterial meningitis (Fassbender et al., 1996). Both conditions are characterised by massive cytokine release (Fassbender et al., 1996), but also by an obvious absence of erythrocytes or platelets that are considered key cells in the pathophysiology of vasospasm following SAH.

The triggers for the pronounced inflammatory host response in the subarachnoid space following SAH are unknown. One mechanism could be complement activation, a strong inflammatory stimulus, by osmotically induced disruption of erythrocytes. This is suggested by in vitro findings of complement mediated haemolysis of erythrocytes in subarachnoid blood clot or by raised CSF concentrations of complement factors in SAH (Fassbender et al., 2001).

Subarachnoid inflammatory mediators could contribute to cerebral vasospasm in several ways. Once in the subarachnoid space, these mediators could easily access the smooth muscle cells of basal arteries from their adventitial surface. This is possible because the surface of pial cerebral arteries, is exceptionally, not confined by collagen or fibroblasts, but is in direct contact with the nourishing CSF. At the smooth muscle cells, these mediators could directly cause vasospasm, although the effects of inflammatory cytokines are still controversial. Cytokines may also modulate vessel tone in an indirect manner, by inducing synthesis of vasoconstrictors such as ET-1, or by induction of the adhesion molecule expression responsible for focal leukocyte recruitment.

Haemostatic systems seem to have a close relationship with delayed vasospasm following SAH. Although its mechanism remains controversial, it is well known that the severity of vasospasm increases with the amount of residual clot (Inagawa et al., 1990; Zabramski et al., 1991). Vasospasm is accompanied by damage to the endothelium, resulting in exposure of the subendothelial connective tissue. Platelets adhere to the connective tissue via the interaction between von Willebrand factor and glycoprotein Ib on the platelet surface, are activated, and are aggregated with each other via the interaction between fibrinogen and glycoprotein IIb/IIIa. The aggregation of platelets results in the generation of thrombin, which converts fibrinogen to fibrin, and subsequently a thrombus is precipitated on the damaged endothelium. It is conceivable that this thrombus may have a close relationship to the appearance of DINDs.

In this study the frequency of DINDs was significantly higher in those patients who presented with hydrocephalus on initial CT imaging. The authors speculated that in these patients, the clearance of subarachnoid clot was delayed because of a disturbance of CSF flow. They suggested that the rise in D-dimer on day 3 following the ictus, could reflect the amount of residual subarachnoid clot, that is the clot that could not be removed at surgery, or cleared with the flow of CSF.

Fibrinogen plays an important role in the platelet aggregation and plasma viscosity. The increased level of fibrinogen itself may be a risk factor for the appearance of DINDs. Thus, hypervolaemic therapy for vasospasm may be effective in decreasing fibrinogen levels, by reducing platelet aggregation and plasma viscosity.

The high level thrombin-antithrombin complex in the patients with DINDs may indicate the precipitation of thrombus on the damaged endothelium. Hence, from a haemostatic standpoint it may be useful for the prevention of DIND to remove the subarachnoid clot extensively as soon as possible and initiate prophylactic therapy, such as hypervolaemic and/or mild anticoagulation therapy, such as low dose heparin administration.

The effects of the heterogeneity of human vascular endothelium may also play a part in the localisation of vasospasm. Although endothelial cells throughout the vasculature share basic structural and functional characteristics, marked differences exist in their morphology and growth characteristics. Vascular endothelial cells release biologically active agents such as nitric oxide and prostacyclin which are powerful vasodilators, and endothelin-1, a potent vasoconstrictor. The balance between dilators and

constrictors released by the endothelium is an important determinant of vascular tone. It is known that the cerebral endothelium differs from peripheral or omental endothelium in its cell proliferation rate, rate of protein synthesis, as well as in its response to various agonists such as α -thrombin, vasoactive intestinal peptide, endothelin-1(Thorin et al., 1997). Peltonen et al(Peltonen et al., 1997) concluded that endothelial dysfunction was causing coagulation cascade activation, and that this subsequently led to DIND. The endothelial cells produce many components of the haemostatic system including vWF and tPA.

Treatment of cerebral vasospasm

The strategies developed in the management of vasospasm include the following. Preventative measures: (a) preoperative management in the form of early surgery and the use of calcium antagonists; (b) intraoperative such as clot removal and/or clot lysis using fibrinolytic agents and minimal manipulation of blood vessels; (c) postoperative management including hypervolaemia and haemodilution. Curative measures: (a) hypertension, hypervolaemia and haemodilution (triple H); (b) intra-arterial vasodilators (pharmacological); (c) transluminal angioplasty (mechanical dilation). Despite all the available measures to prevent and/or treat aneurysmal SAH induced vasospasm, it remains a clinical challenge for neurosurgeons, intensivists, and neuroradiologists(Al Yamany, Wallace, 1999; Fandino et al., 1999).

Several reports from uncontrolled studies, described the resolution of deficits from vasospasm following the elevation of blood pressure, volume expansion, and haemodilution, with improved outcome of vasospasm compared with historical controls(Awad et al., 1987; Kassell et al., 1982;

Levy, Giannotta, 1991; Muizelaar, Becker, 1986). However, the efficacy of hypertension/hypervolaemia/haemodilution (HHH) has not been demonstrated in controlled trials, and studies of cerebral blood flow after initiation of therapy have been equivocal (Fandino et al., 1999). In addition studies have not been performed to determine which component of this therapy (haemodilution versus hypervolaemia versus hypertension) is most important. Only a proportion of the patients with vasospasm respond to H/H/H therapy, with stroke and death rates from vasospasm approaching 15% in the series with the best outcome.

Initiation of H/H/H therapy is associated with significant risk, including cardiac failure, electrolyte abnormalities, cerebral oedema, bleeding abnormalities, and rupture of an unsecured aneurysm. An uncontrolled series suggested that therapy might be more effective if initiated prophylactically before the onset of symptoms (preferably after clipping of the aneurysm). Treatment is usually continued beyond the period of risk for vasospasm or until abatement of vasospasm by clinical or transcranial Doppler parameters.

In patients with severe resistant symptomatic vasospasm unresponsive to HHH therapy, nimodipine or angioplasty, there have been some promising results with barbiturate coma (Finfer et al., 1999). Barbiturates are said to exert their protective effect by decreasing cerebral oxygen consumption, altering regional blood flow and decreasing intracranial pressure.

Haley et al (Haley et al., 1997) conducted a study on 902 patients with SAH into the effect of tirilazad, a non-glucocorticoid 21-amino steroid, which is a potent inhibitor of oxygen free radical induced lipid peroxidation that lacks the side effects of glucocorticoids. Oxygen free radical induced lipid peroxidation

is thought to be a possible factor in the development of vasospasm following SAH. The study showed that the incidence of vasospasm, proportion of patients receiving angioplasty and the outcome at 3 months was the same in treatment and control groups.

Papaverine is a benzyloisoquinoline opium alkaloid that dilates spastic vessels with a half-life of 0.8 hours. The effectiveness of intra-arterial papaverine in the treatment of cerebral vasospasm has not been demonstrated in a randomised clinical trial(Polin et al., 1998). However, an uncontrolled study by Fandino et al(Fandino et al., 1999), showed improvement in angiographic vasospasm and doppler measured mean flow velocities in the main intracranial vessels using a combined protocol and percutaneous angioplasty and intraarterial papaverine infusion. This study in 30 patients with refractive vasospasm showed that mean flow velocity decreased significantly from 135 +/- 48 cm/s to 87 +/- 32 cm/s in the MCA and 110 +/- 37 cm/s to 84 +/- 30 cm/s in the ACA. Early clinical improvement was observed in 73% of patients following treatment and was significantly associated with a good outcome (GOS 4-5).

The effects of volume expansion therapy have not been studied adequately in patients with aneurysmal SAH. At present, there is no sound evidence for or against the use of volume expansion therapy in patients with aneurysmal SAH(Feigin et al., 2000).

Calcium-channel antagonists

In prospective, randomised trials, oral Nimodipine reduced poor outcome due to vasospasm in all grades of patients(Allen et al., 1983). However, the

incidence of symptomatic vasospasm was not affected by Nimodipine treatment, and vessel calibre on angiography was unaffected.

Pickard et al(Pickard et al., 1998) conducted a trial in a randomised, prospective, double-blind population of 199 patients in which nimodipine was given orally in a dose of 60mg every 4 hours, started within 4 days of SAH and continued for 21 days in survivors. The result of the trial was an overall 34% reduction in cerebral infarction and a 40% reduction in poor outcome. In addition, there was a 36% death or severe disability rate in the placebo group, compared to 17% in the treatment group, and 83% good recovery and moderate disability in the nimodipine group, compared to a 64% rate in the placebo group. These differences were significant both clinically and statistically.

The action of nimodipine was initially believed to be vasodilatation but now many scientists believe that the calcium antagonising effect of nimodipine may be the protecting factor against delayed ischaemia(Al Yamany, Wallace, 1999).

Transluminal angioplasty

Zubkov first reported the use of angioplasty for the treatment of vasospasm in 1984(Zubkov et al., 1984). As with cerebral vasospasm, the mechanism by which angioplasty works is not understood. Angioplasty may be effective by disrupting the extracellular matrix in the vessel, which maintains the narrowed state. In patients with vasospasm who clinically deteriorate despite maximal medical therapy, the judicious and aggressive use of timely percutaneous balloon angioplasty in experienced hands can improve short term outcomes(Eskridge et al., 1998). In this retrospective, uncontrolled

study in 50 patients, 61% of patients improved within 72 hours of treatment, 4% died immediately following the procedure, and 2% deteriorated, 33% did not improve. It was found that the longer the vessel was in spasm the more difficult it was to perform angioplasty.

Angioplasty is not without risk. Vessel rupture can occur, and therefore, should not be performed distal to the genu of the MCA, beyond the A1 segment of the anterior cerebral artery, or beyond the P1 segment of the posterior cerebral artery.

The effects of transluminal angioplasty have been summarised as a significant improvement in 60%-80% of patients, often within hours after dilatation, and normal angiographic calibre in nearly all cases, without recurrent vasospasm(Eskridge et al., 1998). Evidence of improved cerebral blood flow by TCD or single photon emission CT correlated with clinical improvement. The complication rate (rupture of vessels or unsecured aneurysm) is approximately 5%. These benefits, and that of intra-arterial papaverine(Kassell et al., 1992) have not been demonstrated in controlled clinical trials.

Blood load and vasospasm

Delayed ischaemic neurological deficits (DIND) due to cerebral artery vasospasm is one of the major complications that cause poor clinical outcome after aneurysmal SAH(Kassell et al., 1990a). It is reported that the severity of neurological deficits from spasm correlates with the amount of basal subarachnoid blood on the admission CT scan(Allen et al., 1983; Fisher et al., 1980; Kasuya et al., 1998). Fisher et al(Fisher et al., 1980) stated that when subarachnoid blood was not detected on the CT scan, or

was distributed diffusely, severe vasospasm was almost never encountered. However, in the presence of subarachnoid blood clots larger than 5 x 3 mm, of layers of blood 1mm or more thick in fissures and vertical cisterns, severe spasm followed almost invariably. They noted an almost exact correspondence between the site of major subarachnoid blood clot and the location of severe spasm. They suggested that blood clots are especially spasmogenic when they lie along or in contact with the major arteries of the circle of Willis, particularly the proximal 9 to 10cm of the anterior and middle cerebral arteries.

Jarus-Dziedzic et al (Jarus-Dziedzic et al., 2000) concluded in a study of 127 aneurysmal SAH patients that the blood load as measured by Fisher grade was significantly correlated with cerebral blood flow velocity measurements using transcranial doppler. These authors found that during the first 21 days following SAH the highest flow velocities were observed in the Fisher grade III and II patients. Lower values were found in patients with massive SAH (group IV) and the lowest flow velocities were recorded in group I patients, that is those without SAH on the original CT scan. In addition the number of days for which the mean flow velocity in the middle cerebral artery was >120cm/s was statically correlated with blood load on CT imaging (I, II and III). In all patients the greatest difference in mean flow velocity measurements were observed between the 9th and 14th day following SAH. The authors suggest that the apparent failure to demonstrate a consistent trend with regard to the grade IV patients could be explained by raised ICP and thus decreased cerebral blood flow in these patients. Similar findings of increased ischaemic deficit and increased doppler flow velocities were also

observed by Grosset et al (Grosset et al., 1994) in relation to increased blood load. Once again however, Fisher grade IV patients were not in agreement with this trend. The authors suggest that this may be explained by the fact that intracerebral and intraventricular blood can be present without significant volumes of subarachnoid blood.

Fujii et al found no significant relationship between the severity of subarachnoid clot and the levels of fibrinogen, D-Dimer, and thrombin-antithrombin complex following SAH (Fujii et al., 1997).

However, because heavy CT blood load also correlates with other prognostic indicators such as, poor condition on admission, coexistent hypertension, age and surgery, the causative relationship between blood load and overall clinical outcome is complex (Grosset et al., 1994).

Clot removal and fibrinolytics

Clinical and experimental evidence has related the severity of cerebral vasospasm to the volume and duration of perivascular thrombosis in the subarachnoid space (Tanaka et al., 1998). Experimental work with clot placement models of vasospasm shows that vasospasm is related to the length of time arteries are in contact with clot (Handa et al., 1987). Kasuya (Kasuya et al., 1998) reported that the patients showing a slower rate of clearance of subarachnoid blood from the basal cisterns had a higher rate of infarction, and higher CSF levels of fibrinopeptide A. Removal of fibrinopeptides from the fibrinogen molecule by thrombin allows the fibrin monomers formed to undergo polymerisation through the production of an insoluble fibrin network. Thrombin is also a potent inducer of platelet aggregation and secretion. This cascade reaction occurs in the subarachnoid

space filled with CSF after SAH. Activation of the coagulation system forms a firm fibrin network entrapping the red cells in the subarachnoid space and influences the persistence of the blood clot in the subarachnoid cisterns(Kasuya et al., 1998). This concept led to the practice of early surgery to ensure extensive clot removal to reduce the incidence and severity of vasospasm(Hosoda et al., 1999; Suzuki et al., 1979). However, no controlled study has demonstrated the effectiveness of this technique in reducing vasospasm, and other authors have found no significant effect of clot removal by surgery(Inagawa et al., 1990). Lysis of subarachnoid thrombus by intracisternal recombinant tissue plasminogen activator is under investigation.

Kodama et al(Kodama et al., 2000) reported the use of cisternal irrigation therapy. In their technique urokinase was used to dissolve residual clot, and ascorbic acid is added to degenerate oxyhaemoglobin, one of the strongest spasmogenic substances, into haem-like products which are non-spasmogenic. They studied the efficacy of this technique in 217 patients with Fisher grade 3 SAH. They reported an extremely low incidence of symptomatic vasospasm in this cohort of 2.8%, with only 0.9% developing clinical sequelae.

Findlay et al(Findlay et al., 1995) reported the results of 100 patients randomised using a single intraoperative dose of recombinant tPA. The results did not show a significant difference in the overall degree of angiographic vasospasm, yet there was a consistent trend toward lesser degrees of vasospasm in the tPA group with no or mild spasm in 68% and

severe spasm in 15%, compared to 42% with no or mild vasospasm and 23% severe vasospasm in the placebo group.

Hosoda and colleagues (Hosoda et al., 1999) examined 32 SAH patients with anterior communicating artery aneurysms, and symmetrical cisternal subarachnoid blood, who had a unilateral pterional approach within 3 days of the ictus. Cerebral blood flow was determined in these patients using single photon emission tomography (SPECT) and related to angiographic vasospasm and blood load on CT imaging. The regional cerebral blood flow (CBF) showed a continuous fall during the first 4 weeks after the ictus, followed by improvement. A significant association between a decrease of cisternal blood after surgery, with the degree of local vasospasm and local CBF values during the spasm stage, was observed in the interhemispheric cisterns, A2 and medial frontal cortex, but not in the sylvian fissure or insular cisterns, M1 or M2, and frontal watershed and temporal cortex. However, they also observed a significant reduction in regional CBF in the vicinity of the surgical route that continued up to 1 month following surgery. They thus concluded that, direct clot removal with early surgery is effective with respect to vasospasm and CBF reduction. However, this potential improvement in CBF with clot removal is masked by brain retraction and oedema, sacrifice of veins, mechanical manipulation of the subarachnoid vessels, and intraoperative induced hypotension, which affects cerebral blood flow adversely at the operative site.

A similar reduction in CBF on the operated side in anterior communicating artery aneurysms was observed by Yoshimoto and colleagues (Yoshimoto et

al., 1999), however they failed to demonstrate any improvement in vasospasm associated with extensive subarachnoid clot removal at surgery.

Coil embolisation and vasospasm

Surgery during the period of maximal vasospasm (4 to 12) days has been traditionally regarded as less suitable, particularly in patients of higher clinical grade. Surgery is therefore often performed early (before) or late (after) the 'spasm period'(Torner et al., 1990). This is largely due to the International Cooperative Study on the timing of aneurysm surgery suggesting that the operative results depended on the time interval between treatment and SAH(Kassell et al., 1990a). Early surgery reduced the risk of rebleeding, but technical results were better when surgery was delayed until at least 10 days after SAH. Overall the advantages of delayed surgery were negated by rebleeding during the preoperative period. Therefore, 6 month outcomes after early surgery were equivalent to those after delayed surgery. The least favourable surgical results were associated with surgery performed between days 7 and 10 after the ictus, that is, during the period of greatest susceptibility to vasospasm.

Endovascular treatment with Guglielmi detachable coils (GDC) is an alternative treatment for acutely ruptured aneurysms that is now well established(Fernandez et al., 1994; Vinuela et al., 1997). In contrast to surgical clipping, the endovascular procedure does not allow removal of the subarachnoid clot. Murayama et al(Murayama et al., 1997) measured the incidence of symptomatic vasospasm after early endovascular treatment of acutely ruptured aneurysms in 69 patients with Hunt and Hess clinical grades between I and III. The 23% incidence of cerebral vasospasm was

comparable with surgical series. In a series of 37 patients(Yalamanchili et al., 1998), preliminary data has suggested that the frequency of cerebral vasospasm may be reduced in those treated by endovascular therapy compared with those treated by direct surgical clipping. Recently, it has been shown in univariate analysis that the vasospasm related ischaemic infarction rate was higher with endovascular treatment versus surgery(Gruber et al., 1998). Charpentier et al's(Charpentier et al., 1999) recent study in 244 patients treated either by surgery or endovascular means did not show any significant difference in mortality, morbidity or the incidence of vasospasm in either of the treatment arms.

A recent report(Wikholm et al., 2000) has suggested that the incidence of ischaemic complications is unaffected when coil embolisation is performed outside or within the period of maximal spasm. However, this compares only a small number of clinical events. Baltsavias et al(Baltsavias et al., 2000) reported on a group of 327 patients consecutively treated with coil embolisation following aneurysmal SAH. They concluded that the interval between endovascular treatment and SAH did not affect periprocedural morbidity rates or 6 month outcomes. They suggested that coil embolisation should be performed as early as possible to prevent rebleeding.

The metabolic response to trauma.

A series of metabolic, water, electrolyte and endocrine changes occur following trauma, sepsis, or surgical operations. These may be divided into 4 phases.

In the injury phase, tissue catabolism occurs.

At the 'turning point', the Na⁺ and K⁺ balance reverses before protein anabolism begins.

During protein anabolism, lean tissue mass is regained.

During fat anabolism, body weight is regained

An alternative nomenclature is that:

A **shock or ebb phase** lasts 1-2 days. Metabolic rate, temperature, and cardiac output are reduced. Catecholamines, glucose, lactate and free fatty acids are increased and insulin secretion is reduced, the patient is in a negative nitrogen balance.

Flow phase occurs from the third day onwards. Metabolic rate, temperature, cardiac output and insulin secretion are increased. Catecholamines, glucose and lactate are slightly increased or normal, and free fatty acids are low. The nitrogen balance becomes positive.

After an injury involving tissue damage, the body mounts a series of responses which are relatively constant irrespective of the nature of the insult. The magnitude of the response though is variable. The principal events which produce signals to initiate this response are fluid loss, afferent sensory nerve output and circulating mediators.

The proteins that increase are often referred to as the acute phase plasma proteins. C-reactive protein, alpha₁-acid glycoprotein, alpha₁-antitrypsin, and fibrinogen are the best known examples. There is evidence to support interleukin I as the mediator of the acute phase protein response.

Those proteins that decrease are albumin, transferrin, prealbumin and retinol-binding protein. The concentration of plasma proteins can decrease as a result of a fall in the rate of synthesis, increased consumption, by

dilution in the intravascular compartment or by loss of protein into extravascular spaces. The last mentioned is probably the most important. Increased vascular permeability is caused by the chemical mediators, histamine and bradykinin, released from the site of injury.

Surgical procedures and coagulation

The development of a hypocoagulable state as a consequence of massive blood loss and transfusion is well established(Hewson et al., 1985). This dilutional coagulopathy occurs after large volume blood loss. However, if the volume lost is only small to moderate, and if no transfusion is performed, the changes in coagulation have not been well studied(Ng, Lo, 1996b).

An in vitro study by Heather et al. was the first to report that a hypercoagulable, in contrast to a hypocoagulable state could develop in association with mild to moderate haemodilution(Heather et al., 1980). More recent in vivo studies have confirmed these findings(Gibbs et al., 1994; Tuman et al., 1987). However, Sharma failed to observe any change in TEG variables in pregnant women given a bolus of Ringers lactate(Sharma et al., 1997).

Tuman et al(Tuman et al., 1987) observed the effects of progressive blood loss on coagulation as measured by TEG. They demonstrated that patients tended to become hypocoagulable after induction of general anaesthesia, when compared to their pre-anaesthetic state. This finding was reflected by a prolongation of R and K values, with a diminution of angle and MA values. However, with moderate blood loss (10-15% estimated blood volume) and at the end of the operation TEG parameters were hypercoagulable compared to the post-anaesthesia values.

However, evidence exists that a hypercoagulable state will develop as a result of the stress of surgery per se (Barrer, Ellison, 1977; Gibbs et al., 1992). The effects of stress, tissue trauma, pain, endogenous catecholamine levels and anaesthesia on global coagulation status are poorly understood. It has been suggested that catecholamine release has a stimulatory effect on platelet aggregation. These factors undoubtedly act as confounding variables in the development of a hypercoagulable state following a surgical procedure or serious illness. From an evolutionary perspective, activation of haemostatic systems could be considered a normal response following surgical aggression, and aimed at preventing excessive bleeding (Arcelus et al., 1995).

Proposed mechanisms have included deficiency of protein C or protein S, high levels of plasminogen activator inhibitor, acute dilution of antithrombin III and consumption of antithrombin III (Nachman, Silverstein, 1993). Physiological concentrations of epinephrine appear to sensitise platelets to other agonists such as ADP. Epinephrine activates platelets in vivo. Platelet volume is increased, thromboxane B₂ formation is enhanced, platelet release is stimulated, and platelet aggregation is enhanced (Hjemdahl et al., 1994). Epinephrine and norepinephrine enhance the fibrinogen binding and P-selectin expression of platelets in the presence of the agonist ADP, both in vivo and in vitro. These platelet responses to agonist stimulation indicate a prothrombotic potential for sympathoadrenal activation in humans (Hjemdahl et al., 1994).

Increased levels of renin, angiotensin, and catecholamines are likely during progressive blood loss. Platelet adhesiveness increases significantly after

angiotensin as well as catecholamine administration(Barrer, Ellison, 1977; Rubegni et al., 1969a). The stress of surgery leads to the release of catabolic hormones, such as cortisol and catecholamines, and local cytokines(1999b). It is likely that surgical stress, tissue trauma (with release of tissue thromboplastin), and elevations in serum catecholamine levels offset any hypocoagulable tendency resulting from haemodilution and loss of coagulation factors during progressive blood loss(Rubegni et al., 1969b; Spagna et al., 1971; Tuman et al., 1987).

Vasopressin (AVP) is concerned physiologically in the maintenance of water balance. However, during periods of stress such as profound nausea, surgical operation, or hypotension, plasma concentrations can transiently rise. The physiological consequences of this are not entirely understood. Grant et al(Grant et al., 1985) have shown in vivo though that this increase is accompanied by a dose response increase in factor VIII and an increase in plasminogen activator. In addition, AVP stimulates blood platelets to aggregate. Grant et al(Grant et al., 1986) observed a similar response in apomorphine induced nausea in control subjects.

A mild to moderate degree of surgical blood loss (10-15%) with haemodilution is associated with the development of hypercoagulability as measured by TEG. No evidence of hypercoagulability was observed at one hour into the operation in the absence of bleeding(Ng, Lo, 1996b). These authors suggested that the hypercoagulability was unlikely to be due to the stress of anaesthesia or tissue trauma per se, as no hypercoagulability was demonstrated unless a degree of haemodilution was also observed one hour into the surgical procedure. Another interesting observation of this study was

that the degree of hypercoagulability as measured by TEG variables, did not progress significantly when the haemoglobin fell from 90% to 85% of the preoperative level. This might suggest that a dilutional hypocoagulable state was evolving as blood loss progressed, counteracting the hypercoagulable state.

Gibbs et al documented the development of hypercoagulable TEG indices in the first 3 days following abdominal aortic surgery(Gibbs et al., 1994). The progressively hypercoagulable state demonstrated with TEG in the first week following open abdominal surgery has been shown to be reversible with heparin and dextran(Arcelus et al., 1995).

However, Benzon et al observed no difference in preoperative, intraoperative and 24 hour postoperative TEG tracings in 120 patients undergoing abdominal and pelvic surgery(Benzon et al., 1994).

Many factors affect the observed changes occurring in coagulation during surgery. These include case mix, use and degree of systemic cooling, operative times, time of day and level of personnel involved(Spiess et al., 1995).

It is important to determine the extent and time course of postoperative hypercoagulability in this group of patients. Only then will it be possible to decide whether clinical trials of antithrombotic therapy are warranted.

It is well established that both vasopressin and adrenaline affect the regulation of both clotting factors and platelets(Grant et al., 1988). Studies have indicated a temporal relationship between increased plasma concentrations of vasopressin and von Willebrand factor (vWF) and factor VIII:C in cardiac surgical patients(Kuitunen et al., 1993). Large molecular

weight multimers of vWF act as glue to overcome high shear forces between circulating platelets and collagen exposed by endothelial injury. In the perioperative period, influences that can raise the concentrations of clotting factors include elevated levels of catecholamines, tissue necrosis factor, interleukin-1, and arginine vasopressin

On the humoral side during trauma or intraoperative stress, the complement system is activated via the alternative pathway. Complement activation plays an important role in shock producing inflammatory mediators because of anaphylatoxin formation (C3a, C4a and C5a). The physiological importance of this has focused on polymorphonuclear neutrophils (PMNs) in terms of adhesiveness, aggregation and degranulation. However, an additional effect of complement activation is the secretion of potent proinflammatory cytokines such as, tumour necrosis factor (TNF) and interleukin 1 (IL-1), and platelet activating factor (PAF) by macrophages. The membrane attack complex (C5b-C9) can release secondary mediators, such as prostaglandin E₂ (PGE₂), leukotrienes, and thromboxane, which are all important in the inflammatory process (Schlag, Redl, 1996). It has been suggested by some authors that the measurement of these complement components can be used to predict the outcome from severe injury (Schlag, Redl, 1996). With the release of the mediators mentioned above, cell damage is produced which that results in a generalised inflammatory response with primary organ damage. Leukocytes and the complement system are triggers during the earliest phase of severe trauma or intraoperative stress, which leads to activation of the complex cascade systems with the release of further tissue-toxic mediators.

During the earliest phase of trauma, adhesion proteins are also involved (P and L-selectin), that are responsible for the adherence of PMNs to endothelial cells. The latter results in damage to the endothelial cell membrane, caused by the release of oxygen radicals and proteases.

During trauma there is early activation of the coagulation system and the release of thrombin via intrinsic and extrinsic pathways. Thrombin stimulates the endothelial cells followed by the expression of adherence proteins (P-selectin). Thrombin also cleaves fibrinogen to fibrin monomers, forming a fibrin clot by polymerisation. These coagulation changes have been associated with the development of acute lung failure, which occurs by the deposition of fibrin in the alveolar space(Schlag, Redl, 1985). This fibrin formation is caused mainly by the transudation of plasma proteins due to the increase in permeability in the capillary region of the lung.

Activation of coagulation via the extrinsic pathway is initiated via factor VII in the presence of tissue factor. Tissue factor is present in many tissues, including brain, monocytes, endothelium and the alveolar interstitial space. It has been suggested that fibrin deposition in connection with depression of fibrinolytic activity, is the basis for the formation of alveolar air block, atelectasis, and disturbances in the ventilation/perfusion ratio(Schlag, Redl, 1996).

The procoagulant stage is also important in the pathological mechanisms in the development of disseminated intravascular coagulation, which results in fibrin deposition in the microvascular system and can be responsible for further organ damage.

Platelet activating factor (PAF) is expressed rapidly by the endothelial cell during shock in response to various stimuli (thrombin, histamine and leukotrienes). This may also be responsible for the earliest adherence of PMNs to endothelial cells (in addition to P-selectin) via PAF receptors. In addition to the cytokines (IL-1 and TNF) this may initiate tissue damage and increase membrane permeability.

Galster et al (Galster et al., 2000) have shown that coagulation and fibrinolysis are significantly enhanced up to 14 days following orthopaedic and abdominal surgery by measuring fibrin monomers and d-dimers.

Coagulation changes and Neurosurgery

Goh et al. used TEG to investigate intraoperative and first day postoperative changes in neurosurgical patients undergoing large tumour resections (>4cm) in 50 patients (Goh et al., 1997). They observed the generation of a hypercoagulable state even in the intraoperative sample (though not in MA) and suggested that this was related to the release of thromboplastic substances from the injured brain. They also demonstrated a significant reduction in PT and APPT on the first postoperative day. This group suggested that there was evidence of reduced activation of coagulation and increased fibrinolysis in those patients who subsequently suffered an intracranial haematoma following surgery compared with the non-haematoma group.

Palmer (Palmer et al., 1995) reports the use of TEG to identify hyperfibrinolysis in a case report of a brain tumour resection and its successful treatment with aprotinin. The demonstration of intraoperative

fibrinolysis by TEG was paralleled by an increase in tPA in the jugular venous samples.

Tsujiguchi and colleagues (Tsujiguchi et al., 1998) report the use of TEG in examining the effect of local anaesthetic infiltration prior to scalp incision. They demonstrated that hypercoagulation in TEG indices was observed following the incision, but that this could be prevented by the prior use of bupivacaine.

Fujii et al (Fujii et al., 1994) performed haemostatic studies on 8 patients undergoing clipping of unruptured intracranial aneurysms in order to assess the influence of intracranial surgery itself. They collected blood before and after induction of anaesthesia, immediately after surgery, 6, 12, 24 hours and, 2, 3, 5, and 7 days after surgery.

Anaesthesia. No difference was observed in any test performed before and after induction of anaesthesia.

Blood coagulation system. The PT was significantly prolonged for 2 days after surgery compared with baseline value. There was no change in APTT. Antithrombin III activity gradually decreased after surgery, reaching its nadir 2 days after surgery (87.3% of baseline), but soon returned to the baseline value. The fibrinogen level started to increase 12 hours after surgery, reached its highest level 2 or 3 days after surgery (191% of baseline), and decreased gradually thereafter. However, its value remained significantly higher than the baseline study until the end of the study. The TAT level rose significantly immediately after surgery, then gradually diminished, and returned to the normal range. Its level, however, remained significantly higher than the baseline value until the end of the study.

Fibrinolytic system. Plasminogen activity rapidly decreased, reached its lowest level 24 hours after surgery (77.3%), and then gradually increased, becoming significantly higher than the baseline value. On the other hand, there was no significant change in α_2 -antiplasmin activity for 3 days after surgery, however, significant increases (116.5%) were observed 5 and 7 days postoperatively. The PAP level became considerably elevated immediately after surgery and, after rapidly returning to baseline, began to rise again 3 days after surgery, significantly exceeding the baseline value. The D-dimer and FDP levels rose significantly immediately after surgery, reaching a peak at 6 hours, then gradually decreased to their lowest value 2 days after surgery, and subsequently increased to a higher level than the initial peak. Their level remained considerably higher than baseline until the end of the study. Both tPA and PAI-1 levels increased significantly initially after surgery, then rapidly decreased and reached baseline values within 24 hours of surgery. No significant elevations of tPA and PAI-1 followed their initial increase.

Platelet system. The platelet count increased slightly immediately after surgery, then gradually decreased, reaching its nadir 2 days after surgery (81%), after which it rapidly increased to become significantly higher than baseline. Both β -thromboglobulin and platelet factor 4 levels rose significantly immediately after surgery, rapidly decreased, and returned to baseline levels within 24 hours. They then gradually increased again, becoming significantly higher 7 days after surgery.

Fibronectin. The fibronectin level gradually decreased after surgery, reached its lowest level (71.8%) 24 hours after surgery, and returned to baseline within 5 days.

Blood cell counts. The white blood cell count (WCC) significantly increased immediately after surgery, peaked at 6 hours (247%), then gradually decreased reaching baseline at 5 days. Although the red cell count, haemoglobin, and haematocrit value did not change immediately after surgery, they gradually decreased, reaching their nadir 3 days after surgery (80%, 77.3% and 76.3%), and remained significantly lower than baseline values until the end of the study.

The above study provides good reference data as most other neurological illness e.g. stroke, brain tumours or trauma, would already be expected to have a haemostatic abnormality before surgery.

The haematocrit gradually decreased by 23.7% probably as a result of haemodilution with intravenous fluids. It is generally accepted that the concentration of platelets, fibrinogen, antithrombin III, α_2 -antiplasmin, and plasminogen, change in parallel with the fall in haematocrit after haemodilution (Fujii et al., 1994). Thus, haematocrit is an important parameter in assessing changes in haemostatic systems.

Heesen et al (Heesen et al., 1997a) evaluated the changes in coagulation and fibrinolysis in 15 patients undergoing elective brain tumour resection via a craniotomy, who were not treated with heparin. They observed a significant increase in TAT at 1 hour, with a maximum 3 hours after the beginning of surgery. Returning to baseline on the first postoperative day. A similar time course for F1 and 2 (also indicating coagulation activation) was also

observed. These authors suggested that the dramatic increase in TAT, compared with the observations of Fujii(Fujii et al., 1994) in aneurysm patients, could possibly be explained with the release of thromboplastin from the operative site were more brain tissue was transgressed in brain tumour resection. D-dimer (activation of fibrinolysis) increased throughout the study period and was at a maximum on the first postoperative day. Heesen et al(Heesen et al., 1997a) also measured PT and APPT at each time point and failed to observe a significant change in these coagulation tests.

It is well known that increases in the concentration of many haemostatic substances are observed shortly after chemical, inflammatory, or physical stimulation, i.e. the acute phase reaction, mainly due to the interaction of cytokines. The acute phase reaction must be considered in evaluating postoperative changes in haemostatic systems.

Iberty and colleagues(Iberty et al., 1994) examined coagulation changes in 20 neurosurgical patients undergoing brain tumour (mostly benign) resection. Factor VIII, IX, vWF and AVP were significant increased at the end of surgery and postoperatively. Bleeding times and APPT were significant shortened from the time of incision, intra and postoperatively. Serum osmolality was significantly elevated in the neurosurgical patients from the time of incision. Both factor VIII and vWF are known to be stimulated by AVP and increased to the same extent. However, this group of patients were fluid restricted and haemoconcentrated. It is possible that these measures were responsible for the reduction in bleeding times and APPT.

Goobie et al. have suggested that hypercoagulation occurs in children undergoing resective brain procedures. In 25 children they suggested that

TEG demonstrated hypercoagulation was proportional to the amount of brain resected, perhaps related to thromboplastin release from injured brain(Goobie et al., 1999).

The effects of anaesthetic agents on coagulation

There is little evidence in this field. However, some studies have shown that TEG variables tend to have a more hypercoagulable profile when comparing general with regional anaesthesia for the same surgical procedure, such as caesarean section(Sharma, Philip, 1997). This study showed accelerated clot formation but no change in final clot strength. However, Gibbs et al showed no difference in general compared with general plus regional anaesthetic for abdominal aortic surgery(Gibbs et al., 1994).

Tuman et al(Tuman et al., 1987) demonstrated patients to become hypocoagulable after induction of general anaesthesia compared to their pre-anaesthetic state using TEG variables. The authors suggested that, this may be explained by a decreased level of stress and lower serum catecholamine levels, than compared to the unanaesthetised preoperative state. Additionally, some general anaesthetics have been shown to have direct effects on blood coagulation, especially platelet function. Propofol appears to have little effect on coagulation but significantly increases fibrinolysis when assessed in vitro with TEG analysis(Kohro et al., 1999).

The effects of intravenous fluids on coagulation

The crystalloid versus colloid debate continues as to which fluid provides the best means of fluid resuscitation(Alderson et al., 2000; Hillman et al., 1997). There is extremely strong evidence that the different crystalloid and colloid solutions used for intravenous fluid replacement have very definite and

repeatable effects on coagulation and fibrinolysis. A full discussion on this topic can be found in experimental study 6.

Gender differences and haemostasis

Profound differences in haemostatic responses at rest and in response to a specific systemic stress have been reported as a direct result of gender. A full discussion of this topic can be found in control study 2.

Immobilisation

Patients with SAH are confined to strict bed rest following the ictus, and for some considerable time following treatment. During immobilisation there is venous stasis due to a lack of the calf muscle pump effect as well as venous stagnation in intra-abdominal organs(Inci et al., 1995).

Risk factors for thrombosis

Virchow postulated 3 groups of causes of thrombosis; reduced blood flow, changes in the vessel wall, and changes in blood composition(Virchow, 1856). However, clinically thrombosis is multifactorial with genetic, environmental and behavioural risk factors(Rosendaal, 1999)(Table 20).

Deep vein thrombosis (DVT) and pulmonary embolus (PE) are appreciable causes of morbidity and mortality in surgical patients(Bynke et al., 1987; Iberti et al., 1994; Lazio, Simard, 1999). Those patients undergoing neurosurgery constitute one of the high risk groups for the development of venous thromboembolism(Hamilton et al., 1994; Inci et al., 1995; Stephens et al., 1995). Factors responsible for this include long operative times, release of brain thromboplastic substances during surgery, prolonged postoperative bed rest or immobile limbs due to neurological deficit, high

dose glucocorticoid treatment and dehydrating osmotic treatment(Frim et al., 1992).

Deep vein thrombosis (DVT) in the calf veins of patients undergoing neurosurgery was estimated at an incidence of 19-50%(Agnelli, 1999), as measured by radiolabelled fibrinogen testing. Clinically evident DVT occurred in 2.3% of 523 patients admitted to a combined neurology-neurosurgery service, 1.8% of these patients developed pulmonary embolus diagnosed by lung scan and angiography(Agnelli et al., 1998). Mortality from pulmonary embolus ranges from 9 to 50%(Macdonald et al., 1999). The safety of low dose subcutaneous heparin for DVT prophylaxis in a broad range of neurosurgical procedures has been demonstrated(Agnelli et al., 1998; Frim et al., 1992; Hamilton et al., 1994; Macdonald et al., 1999), but not its effectiveness. The safety of the newer, more effective direct thrombin inhibitors, such as recombinant hirudin(Eriksson et al., 1996; Eriksson et al., 1997) has not been shown in a neurosurgical population.

Other prothrombotic conditions

Factor V Leiden mutation, or hereditary resistance to activated protein C has a prevalence of 5% in white persons, with some geographical variation, but is virtually absent in Asian and African populations(Dahlback et al., 1993; Rees et al., 1995). The presence of other well recognised prothrombotic defects, such as protein C, protein S and antithrombin, have a much lower prevalence(Vandenbroucke et al., 2001). These defects all increase the risk of thrombosis and have been shown to be additive to the risk of the oral contraceptive pill and HRT in females.

High levels of factor VIII are linked to blood groups other than O, which accounts in part for the observation that having a blood group other than O, increases the risk of venous thrombosis by a factor of about 1.6 in general, and by a factor of 3.3 among users of oral contraceptives(Jick et al., 1969).

Acquired	Inherited	Mixed/unknown
Age	Antithrombin deficiency	Hyperhomocysteinaemia
Previous thrombosis	Protein C deficiency	Increased factor VIII
Immobilisation	Protein S deficiency	APC resistance
Major surgery	Factor V Leiden	
Malignancy	Prothrombin 20210A	
Oral contraceptives	Dysfibrinogenaemia	
Hormone replacement therapy		
Antiphospholipid syndrome		
Myeloproliferative disorders		
Polycythaemia vera		

Table 20. Risk factors for venous thrombosis

Experimental study 1

Serial changes in standard haematological and coagulation indices following SAH

Introduction

A standard full blood count generates a series of haematological indices. The Haemoglobin concentration (Hb) is a measure of the amount of this protein molecule, specialised to carry oxygen, in a fixed volume of blood. The haematocrit (Hct) or packed cell volume is a measure of the volume of the cells in a fixed volume of blood. The red cell count (RCC), white cell count (WCC), and platelet count (plt), are a measure of the number of red cells, white cells, and platelets respectively, in a litre of blood in that individual (Table 21).

The prothrombin time (PT) measures the extrinsic system (factor VII) as well as factors common to both systems (factors X, V, prothrombin and fibrinogen). Tissue thromboplastin (brain extract) and calcium are added to plasma. The normal time for clotting is 10-14 seconds. The international normalised ratio (INR) is the ratio of the PT to a control PT expressed as a fraction. The activated partial thromboplastin time (APTT) measures the intrinsic system factors VIII, IX, XI and XII in addition to factors common to both systems. Three substances; phospholipid, a surface activator (e.g. kaolin), and calcium are added to plasma. The normal time for clotting is 35-45 seconds.

	Male	Female
Hb (g/dl)	14.0 - 17.7	12.2 - 15.2
Hct (PCV) (l/l)	0.42 - 0.53	0.36 - 0.45
RCC ($\times 10^{12}/l$)	4.5 - 6.0	3.9 - 5.1
WCC ($\times 10^9/l$)	4.0 - 11.0	
Plt ($\times 10^9/l$)	150 - 400	
PT (s)	10 -- 14	
APPT (s)	35 -- 45	

Table 21. Reference ranges of normal haematological and coagulation parameters.

Methods

This was a prospective, observational study on 67 consecutive patients admitted with a primary diagnosis of SAH to the National Hospital for Neurology and Neurosurgery, London, UK, from January 2000. The diagnosis of SAH was confirmed by CT, lumbar puncture or cerebral angiography.

There were 24 males, median age 47.5 years (25-75) and 43 females, median age 53 years (23-80) (Figure 23). Women were preferentially represented in this sample (1.8:1). Using the World Federation of Neurosurgeons system for grading the admission of SAH patients (Appendix I), 37 of the patients were grade 1, 11 grade 2, 4 grade 3, 10 grade 4, and 5 grade 5 (Figure 24 and Table 22). The cause of the SAH was a ruptured cerebral aneurysm in 56 patients, an arteriovenous malformation in 2, the angiogram was negative in 6, and the cause was undetermined in 3 patients. Blood was taken via a standardised technique, previously described in control study 1 for venous sampling. Arterial sampling was performed through a heparinised arterial line. The technique involved removing the dead space from the line prior to sampling. In this way heparin contamination of the sample is avoided.

The day of ictus (Day 0) was taken to be the day of onset of severe symptoms (e.g. headache, neck pain, neurological deficit, confusion, collapse). Blood was taken at 4 time periods (<48hours, 4-5, 9-10 and 15-16 days) following the ictus depending on the day of hospital admission, and on regular intervals during the hospital stay. If admission was not within one of the time periods above, a sample was taken on the day of admission to the

National Hospital. In addition, a sample was taken at 3 months from the ictus. The time periods were chosen to reflect the practicality of referral speed to a tertiary hospital, the most frequently documented onset time of cerebral vasospasm (4-9days), and the first routine outpatient appointment following discharge (3 months).

A standard protocol of 3 litres of normal saline, 60mg of nimodipine every 4 hours for 21 days and simple codeine based analgesia was followed for all patients.

Studies on haemostasis are often difficult to analyse because of wide inter-patient variability and a wide range of normal values(Chalkiadis, Gibbs, 1996). In this study, we have attempted to reduce inter-patient variability by collecting control (3 month) and test samples from the same patient i.e. each patient is effectively acting as their own control.

At each time point a 4 ml EDTA Vacutainer™ (Ref. 367839) sample was taken for a FBC (Hb, RCC, WCC, Plt and Hct), and a Vacutainer™ (Ref. 367914) 0.105M (3.2%) buffered sodium citrate tube for PT, INR, and APTT.

Arterial sampling

Previous control studies have shown no statistical difference in TEG analysis performed on arterial and venous samples from the same patient sequentially(Spiess et al., 1987).

In addition, to exclude any contamination effect from the heparinised arterial line, we compared the citrated sample collected after removing the dead space using Heparinase modified TEG and the unaltered sample, tested sequentially through two channels. The technique of heparinase modified TEG removes the effect of any heparin present, and allows the underlying

coagulation pattern to be observed. The output of the two channels can then be compared and the effect of any heparin present can be assessed. Previous studies have shown that heparinase reverses the effect of heparin, but has no direct effect on the TEG profile(Gibbs, Bell, 1998; Tuman et al., 1994). We observed no difference between the unaltered citrated sample and the heparinase modified test, showing that there was no heparin contamination of the sample. Thus, the technique used to remove the dead space from the line was effective. Moreover, our control data had shown that there was no systematic difference between the two TEG channels.

Anaesthetic and fluid replacement protocol for patients undergoing intracranial surgery

Patients received fentanyl (3-4 micrograms/Kg), propofol (3 mg/Kg) and atracurium (0.5 mg/Kg) on induction. Maintenance consisted of ventilation with 50% oxygen and air, with 0-2% sevoflurane and a remifentanyl infusion as required to maintain blood pressure at preoperative levels. All patients received 1 mg/Kg of morphine at the end of anaesthesia for postoperative pain, and bolus doses intravenously as required. Regular paracetamol (1 g every 6 hours) commenced 6 hours postoperatively.

Intraoperative fluids consisted of 1.5-2 litres of normal saline, with gelofusine to maintain a CVP of 10-12 mm Hg.

Thromboembolism prophylaxis

All SAH patients had graduated pressure TED[®] stockings for the duration of their inpatient hospital stay. In addition, intermittent external pneumatic calf compression was used intraoperatively in all surgically treated

patients(Bynke et al., 1987). No aspirin or subcutaneous heparin prophylaxis was given to any patient.

Corticosteroids

Corticosteroids are often administered preoperatively to neurosurgical patients. It has been suggested that hypercoagulability secondary to steroid treatment may act synergistically to precipitate intravascular coagulation. This condition can also increase the tendency to thromboembolism(Inci et al., 1995).

No corticosteroids were given to any patient in the study as part of the routine management of cerebral swelling and oedema.

Exclusions

Patients with a known coagulation disorder, on anticoagulation medication, taking the oral contraceptive pill, or in whom the day of ictus could not be accurately stated, were excluded. Any patient with a history of trauma precipitating the event was excluded. Pregnant and paediatric (<16 yrs) patients were also excluded.

Ethical approval

All studies were approved by the joint medical ethics committee of the Institute of Neurology and University College Hospitals Trust (**Ref. No. 99/N037**).

Consent

All patients or their relatives provided informed consent for the study (Appendix IV).

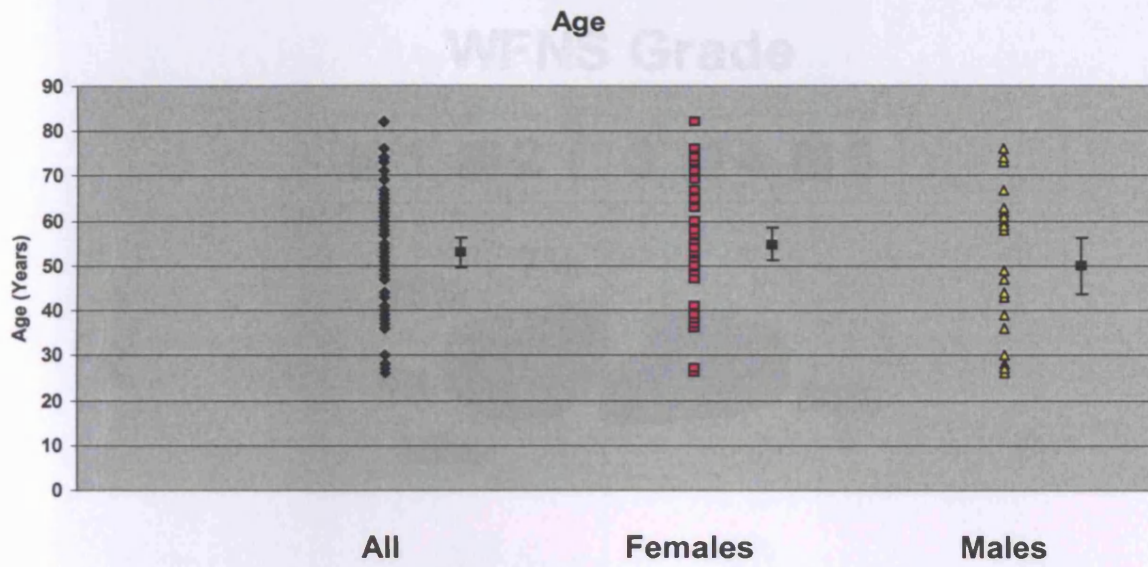


Figure 24. Pie chart demonstrating the percentage of SAH patients in the study stratified in each WFNS grade.

Figure 23. Scatter plot of the age distribution in SAH patients showing mean and 95% confidence intervals.

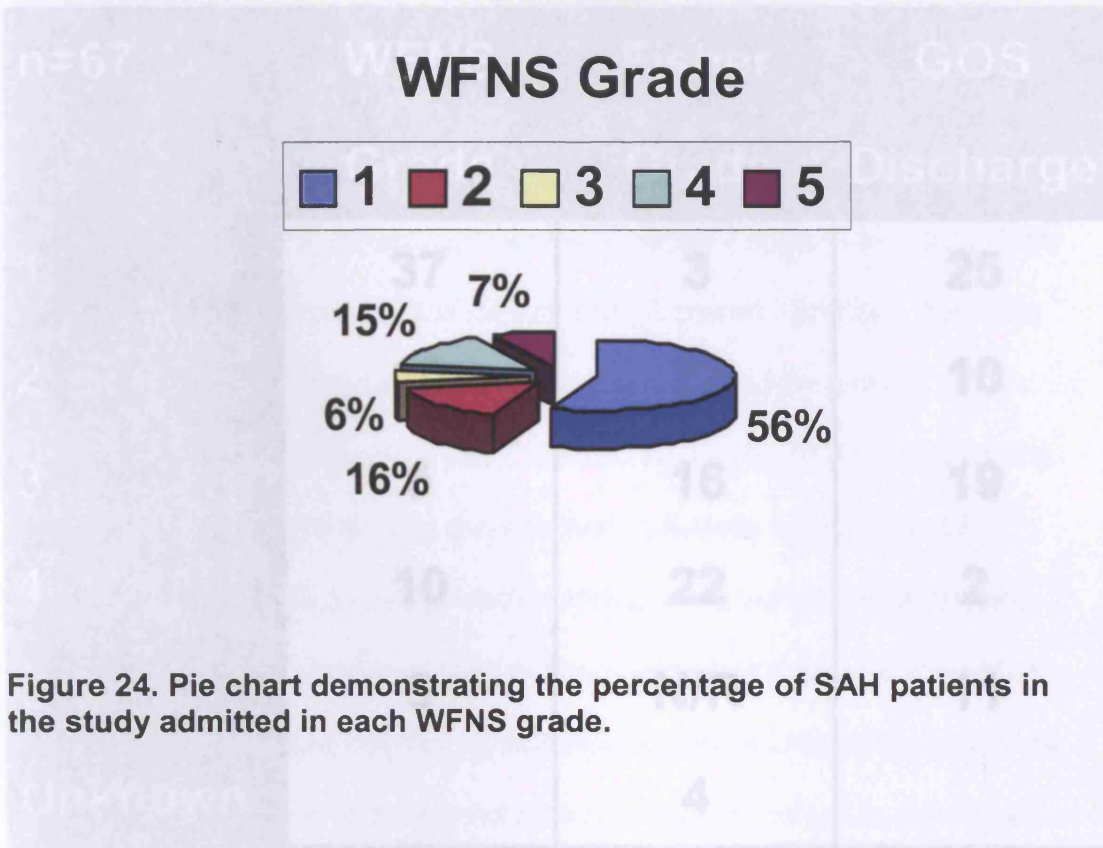


Figure 24. Pie chart demonstrating the percentage of SAH patients in the study admitted in each WFNS grade.

Table 22. Summary table of admission and discharge grades for SAH patients.

World Federation of Neurosurgeons Grade (WFNS) – Appendix I
 Fisher grade – Appendix II
 Glasgow outcome score (GOS) – Appendix V
 N/R – not relevant

n=67	WFNS Grade	Fisher Grade	GOS Discharge
1	37	3	25
2	10	22	10
3	5	16	19
4	10	22	2
5	5	N/R	11
Unknown		4	

Table 22. Summary table of admission and discharge grades for SAH patients.

World Federation of Neurosurgeons Grade (WFNS) – Appendix I

Fisher grade – Appendix II

Glasgow outcome score (GOS) – Appendix V

N//R – not relevant

Results

The results show that there were differences in some of the tests depending on whether the measurement was taken soon after the SAH, or 3 months later. In the coagulation tests, there was a statistically significant difference in the PT which was shortened initially following the SAH compared to the tests performed at 3 months. There was no difference in the value for APTT (Table 23, and Figures 25 and 26). Even though PT was shortened, both means lay

Statistical analysis

The first analysis involves a comparison of the 3-month measurement of PT, APTT, WCC, Hb, RCC, Hct, and plts, with the first ever measurement following the SAH, if that measurement was within 7 days of the ictus. This analysis compared the results of 34 patients. A paired t-test and Wilcoxon signed rank tests, were used to compare these measurements.

The mean rate of change of PT, INR, APTT, WCC, Hb, RCC, Hct, and plts was calculated over the first 21 days following SAH in each of the 67 patients. The average slopes of each variable were then compared using firstly a one-sample t-test, and then subsequently a Wilcoxon signed rank test. The latter test was chosen on statistical advice as not all the data was normally distributed, and the signed rank test is more robust in this regard. For some of the t-tests the log of the value has been taken prior to analysis. This is because t-tests assume a normal distribution in the data, and taking logs means that the assumption of normality holds more closely. The log has no effect on the Wilcoxon signed rank test, which returns the same result, whether or not logs are used.

A p value $p < 0.05$ was taken to be significant.

Results

The results show that there were differences in some of the tests depending on whether the measurement was taken soon after the SAH, or 3 months later. In the coagulation tests, there was a statistically significant difference in the PT which was shortened initially following the SAH compared to the tests performed at 3 months. There was no difference in the value for APTT (Table 23, and Figures 25 and 26). Even though PT was shortened, both means lay

comfortably within the reference range. The mean after the SAH was 11.13 seconds, and 3 months later it was 12.81 seconds.

It can be seen from Table 25, and Figures 25 and 26, that there is no change in PT, INR or APTT in the first 21 days following the ictus, and that all means lie within the standard reference ranges.

There are statistically significant differences in WCC, RCC and Hct, between the first and 3 month values. The WCC rises acutely initially after the SAH, and then remains elevated but does not change over the next 21 days (Tables 24 and 26, and Figure 27). The mean WCC value changes from 11.94 to 7.32 ($\times 10^9 /l$) from the first to the 3 month assessment.

The RCC falls acutely and then continues to fall at a statistically significant rate of $0.005 \times 10^{12} //day$ for the first 21 days following the ictus (Tables 24 and 26, and Figure 28). The Hct also falls acutely and then continues to fall at a statistically significant rate of $0.005 l/l/day$ for the first 21 days (Tables 24 and 26, and Figure 30).

Although the haemoglobin concentration is lower following the SAH than at 3 months, this does not reach statistical significance. However, Hb does fall significantly over the first 21 days at a rate of $0.17 g/dl/day$ (Tables 24 and 26, and Figure 29).

The platelet count shows an interesting change. There is no statistically significant difference between the first and 3 month value (Table 24).

However, it can be seen from Figure 31, that the platelet count appears to fall initially, and then rises acutely up to 21 days at a rate of $14.15 \times 10^9 //day$ (Table 26 and Figure 32).

n=34	First measurement		3 month measurement		P value	
	Mean	SD	Mean	SD	t-test	Wilcoxon
Log PT	2.41	0.02	2.55	0.024	<0.0001	<0.0001
Log APPT	3.35	0.125	3.36	0.822	0.49	0.4

Table 23. PT and APTT comparing the first assessment following the SAH with the value in the same 34 patients 3 months later.

P values are shown using a paired t-test, and Wilcoxon rank sum tests.

n=34	First measurement		3 month measurement		P value	
	Mean	SD	Mean	SD	t-test	Wilcoxon
Log WCC	2.48	0.31	1.99	0.28	<0.0001	<0.0001
RCC	4.37	0.57	4.66	0.56	0.002	0.003
Hb	13.27	1.76	13.76	1.33	0.21	0.06
Hct	0.39	0.05	0.42	0.04	0.01	0.003
Plts	244.9	82.42	250.82	69.63	0.43	0.11

Table 24. WCC, RCC, Hb, Hct, and Plts comparing the first measurement made following the SAH with the value in the same 34 patients 3 months later.

P values are shown using a paired t-test, and Wilcoxon rank sum tests.

Log INR	0.0002	0.002
Log APTT	0.0019	0.004

Table 25. The mean rate of change per day of INR, Hct, and APTT, in the first 21 days following SAH.

SD = Standard deviation

SE = Standard error

P values are shown using a single sample t-test, and Wilcoxon rank sum tests.

n=67	Mean	SD	95% confidence interval
Log PT	0.0033	0.016	(-) 0.0005 to 0.0072
Log INR	-0.00002	0.015	(-) 0.003 to 0.003
Log APTT	0.0019	0.031	(-) 0.006 to 0.010

n=67	Mean	SE	p value	
			t-test	Wilcoxon
Log PT	0.0033	0.002	0.09	0.32
Log INR	-0.00002	0.002	0.99	0.19
Log APTT	0.0019	0.004	0.64	0.41

Table 25. The mean rate of change per day of PT, INR and APTT, in the first 21 days following SAH.

SD = Standard deviation

SE = Standard error

P values are shown using a single sample t-test, and Wilcoxon rank sum tests.

Table 26. The mean rate of change per day of WCC, RBC, Hb, Hct and Plla, in the first 21 days following SAH.

SD = Standard deviation

SE = Standard error

P values are shown using a single sample t-test, and Wilcoxon rank sum tests.

n=67	Mean	SD	95% confidence interval
Log WCC	(-) 0.0015	0.035	(-) 0.01 to 0.007
RCC	(-) 0.053	0.073	(-) 0.07 to (-) 0.036
Hb	(-) 0.171	0.233	(-) 0.23 to (-) 0.12
Hct	(-) 0.005	0.007	(-) 0.006 to (-) 0.003
Plts	14.15	12.53	11.23 to 17.07

n=67	Mean	SE	p value	
			t-test	Wilcoxon
Log WCC	(-) 0.0015	0.004	0.64	0.9
RCC	(-) 0.053	0.008	<0.0001	<0.0001
Hb	(-) 0.171	0.027	<0.0001	<0.0001
Hct	(-) 0.005	0.0008	<0.0001	<0.0001
Plts	14.15	1.47	<0.0001	<0.0001

Table 26. The mean rate of change per day of WCC, RCC, Hb, Hct and Plts, in the first 21 days following SAH.

SD = Standard deviation

SE = Standard error

P values are shown using a single sample t-test, and Wilcoxon rank sum tests.

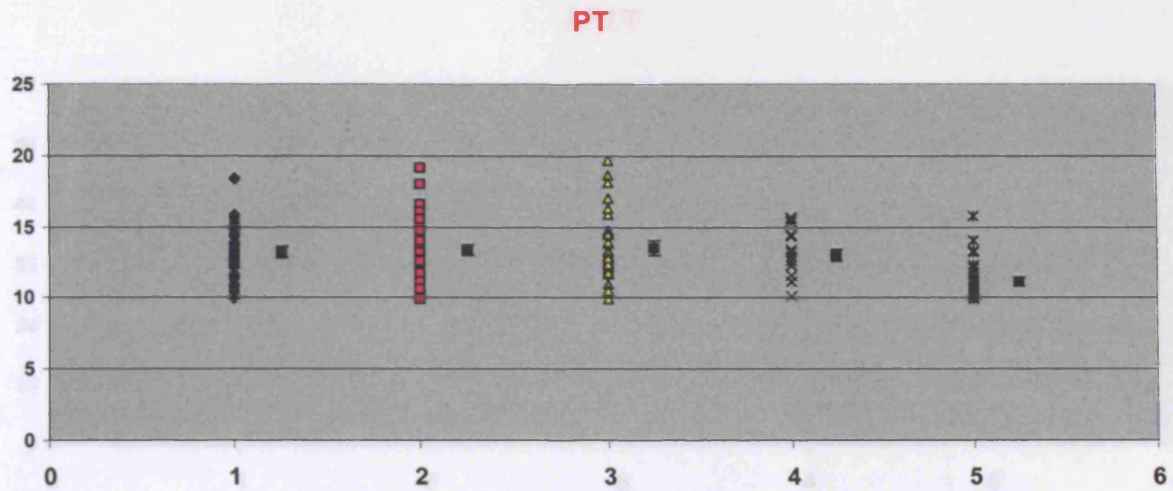


Figure 25. Scatter plot of prothrombin time (PT) for each of the first four weeks following SAH (1-4).

The mean PT value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

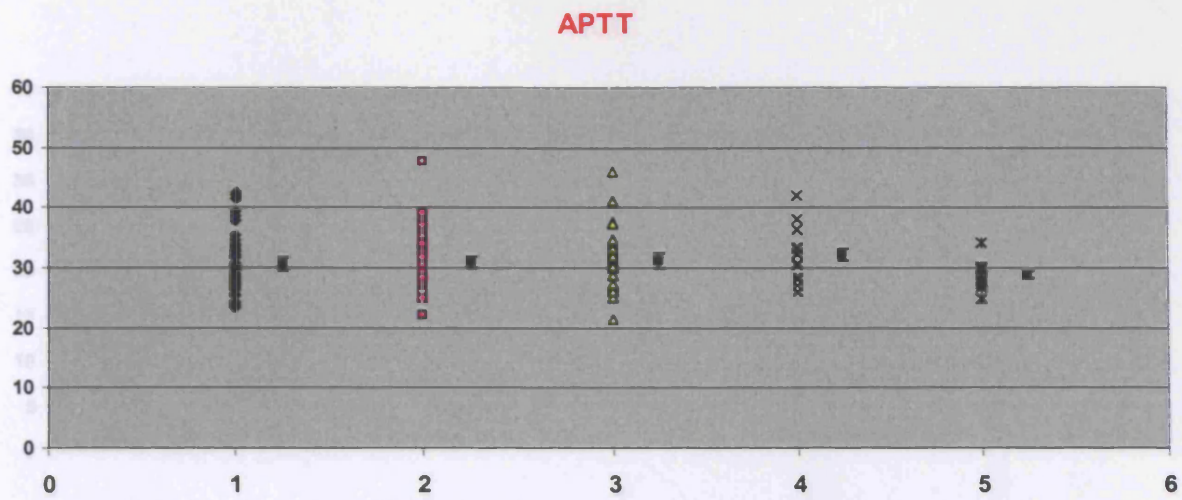


Figure 26. Scatter plot of activated partial thromboplastin time (APTT) for each of the first four weeks following SAH (1-4).

The mean APTT value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

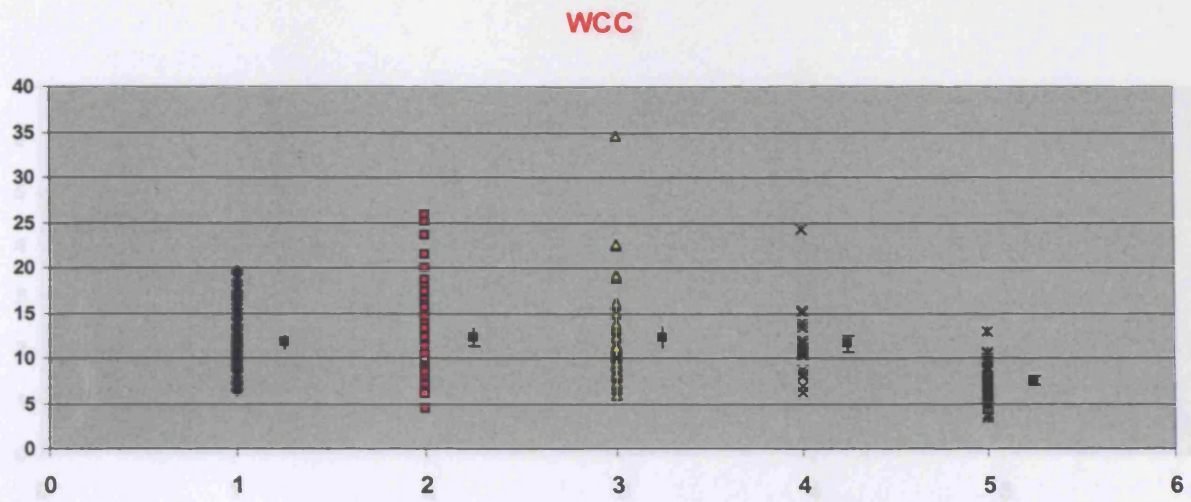


Figure 27. Scatter plot of white cell count (WCC) for each of the first four weeks following SAH (1-4).

The mean WCC value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

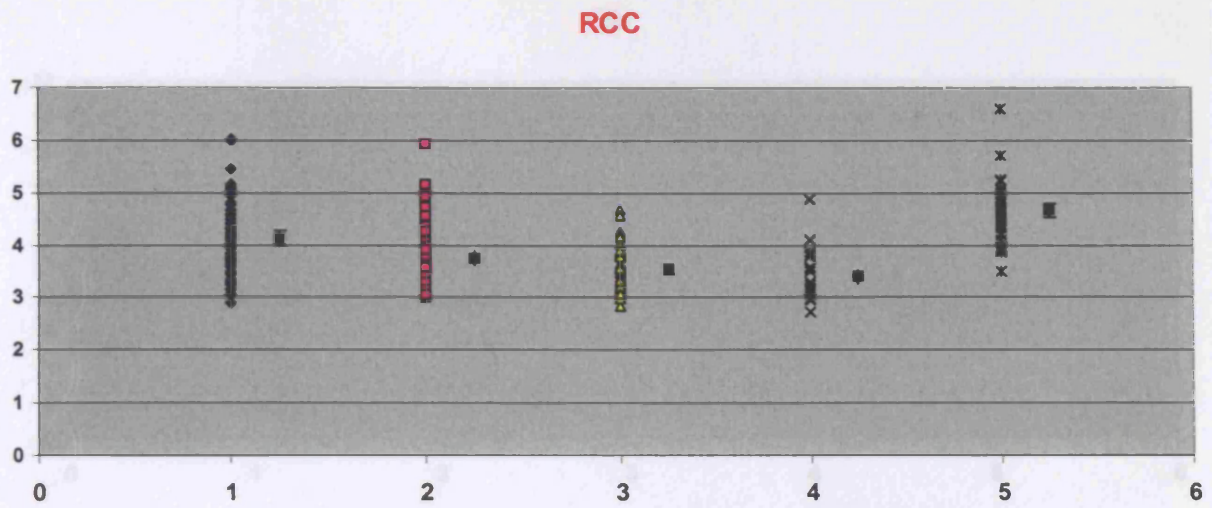


Figure 28. Scatter plot of red cell count (RCC) for each of the first four weeks following SAH (1-4).

The mean RCC value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

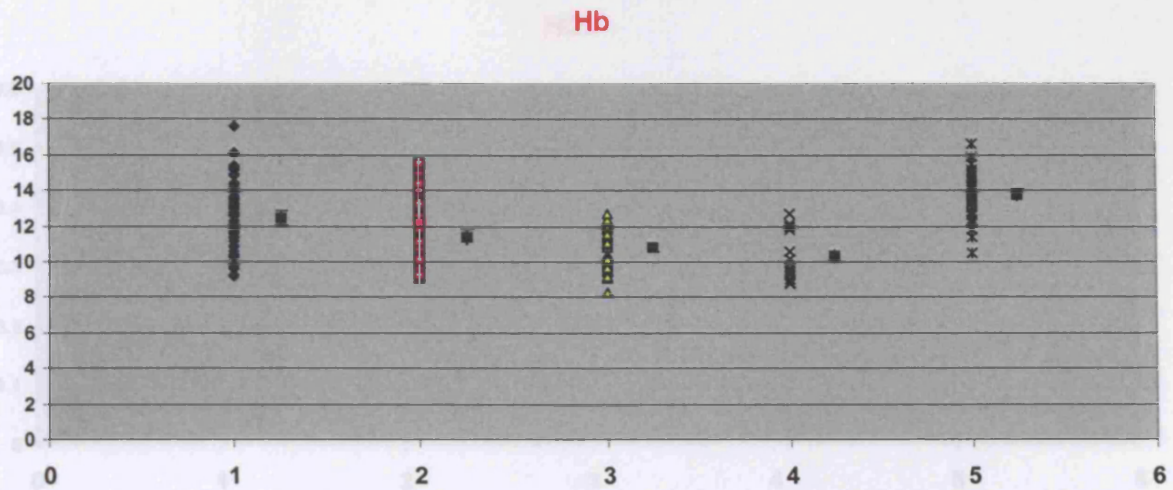


Figure 29. Scatter plot of haemoglobin concentration (Hb) for each of the first four weeks following SAH (1-4).

The mean Hb value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

HCT

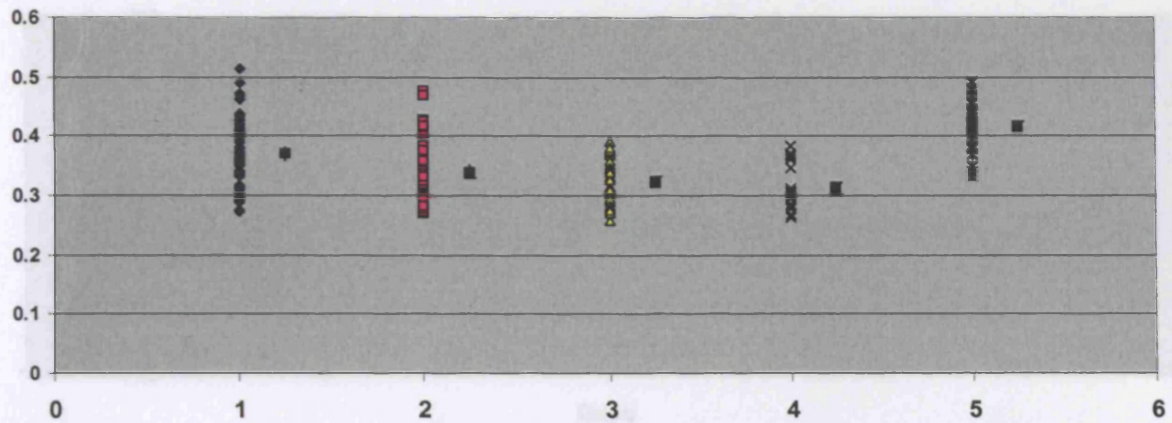


Figure 30. Scatter plot of haematocrit (Hct) for each of the first four weeks following SAH (1-4).

The mean Hct value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

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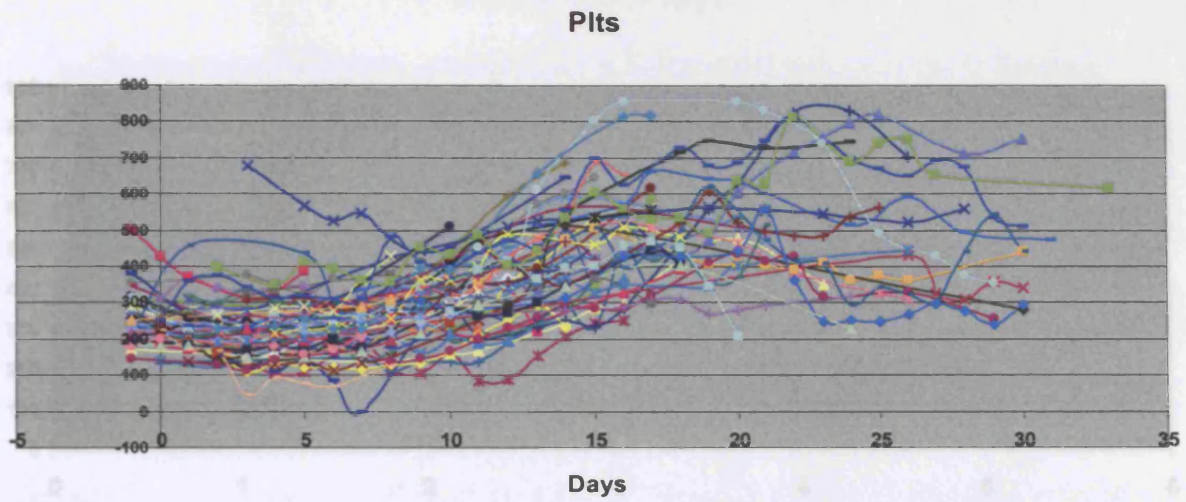


Figure 31. Scatter plots of the change in platelet count (Plts) in each patient following a SAH.

The 3-month reading for each patient is shown to the left of the X-axis. It can be seen that there is a trend for an initial fall after the ictus, followed by a significant rise.

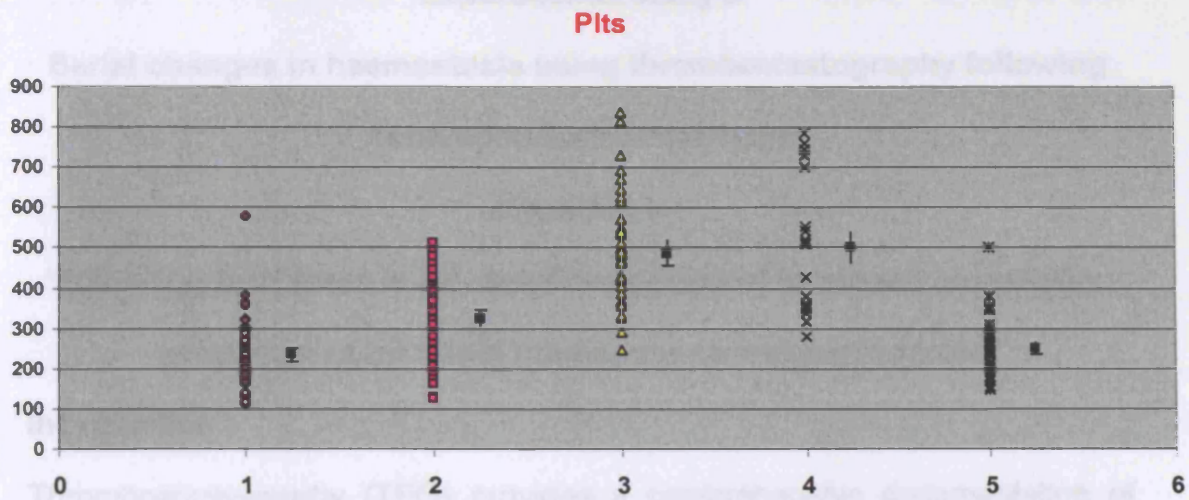


Figure 32. Scatter plot of platelet count (Plts) for each of the first four weeks following SAH (1-4).

The mean Plt value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

Experimental study 2

Serial changes in haemostasis using thromboelastography following subarachnoid haemorrhage

Hypothesis

**'Following SAH there is an undefined period of increased coagulation
which if present could predispose to ischaemic stroke'**

Introduction

Thromboelastography (TEG) provides a comprehensive documentation of the overall clotting process, from the formation of the first fibrin strands to clot dissolution, thus time-profiling the viscoelastic properties of the blood clot.

TEG has never previously been used to investigate haemostasis in SAH.

In SAH, if rebleeding is related to hypocoagulability which can be measured by bench techniques, then pharmacological manipulation of coagulation could be focussed on the administration of antifibrinolytic agents to those who have not entered the period of fibrinolytic shutdown. A reduction of the early rebleeding risk would allow for delayed surgery and significantly impact on the outcome of haemorrhage. Conversely, if the period of hypercoagulability can be correlated to the period when patients are at greatest risk of DIND, then early surgery followed by anticoagulation may impact on the outcome.

Methods

As for experimental study 1.

A 3.6ml volume of venous or arterial blood was sampled through a 19-gauge needle, into a Vacutainer™ (Ref 367914) 0.105M (3.2%) buffered sodium citrate test tube. Blood was taken via a standardised technique, previously

described in control study 1 for venous sampling. Arterial sampling was performed through a heparinised arterial line.

A TEG profile performed at 37°C was obtained at each time point.

Statistical analysis

We have looked at time trends in 6 different TEG variables, namely, R (secs), K (secs), Angle (deg), MA (mm), tMA (secs), and G (dyn/cm²). The first analysis involves a comparison of the 3 month measurement, with the first ever TEG measurement following the SAH, in the 40 patients in which a 3 month measurement was available. A paired t-test and Wilcoxon signed rank tests, were used to compare these measurements. The Wilcoxon signed rank test was used in addition because some of the data for tMA and G was not normally distributed.

The next analysis involved calculating the rate of change of each of the TEG variables over time for the first 21 days following the SAH. This was calculated by regressing each clotting parameter in turn on the time since SAH, for each individual separately. A single sample t-test was used to see if the mean rates of change differed significantly from zero. A significance level of $p < 0.05$ was taken.

Results

The results show that there were differences in all the TEG parameters (excluding R), depending on whether the measurement was taken soon after the SAH, or 3 months following the SAH (Table 27 and Figures 33 to 38).

The R time was unchanged (Figure 33). However, there was a statistically significant reduction in K time and tMA (Figures 34 and 37). There was a statistically significant increase in Angle, MA and G (Figures 35, 36 and 38).

The mean rate of change of each of the TEG parameters over the first 21 days following the SAH is shown in Table 28. The table also shows the standard deviation of the mean rate of change, and the 95% confidence interval of the mean rates of change, averaged across all individuals.

It can be seen that again there is no change in R time over the first 21 days (Figure 33). However, the mean K time is decreasing by 3.22 seconds/day (Figure 34). The Angle is increasing by 0.74 degrees/day (Figure 35). The MA is increasing by 0.78 mm/day (Figure 36). The tMA is decreasing by 26.70 seconds/day (Figure 37). Finally, G is increasing by 524 dyn/cm²/day (Figure 38). In Figures 33 to 38, the mean and 95% confidence intervals for the mean are demonstrated for the fourth week following the SAH. This is to give an impression of the trend in these variables over a longer time scale. However, because there are much fewer data points at this time, they have not been included in the statistics of the rate of change in the TEG variables, which has been confined to the first 21 days.

It can be observed that for each TEG variable, except R time, the data demonstrates a tendency for an increasing hypercoagulable state for the first 21 days following SAH.

There was no evidence of an age trend in any of the rates of change ($p > 0.2$ in all cases).

	First measurement of clotting parameters taken after SAH (n=40)		Measurement of clotting parameters taken at least 3 months after the SAH (n=40)		P-value for comparison (n=40)	
	Mean	Standard devn	Mean	Standard devn	paired t-test	Wilcoxon signed rank
R (seconds)	484	95.6	477	122	0.72	0.91
K (seconds)	98.2	27.8	122	42.9	0.0007	0.0004
Angle (degrees)	67.5	6.01	62.5	7.80	0.0004	0.0003
Ma (mm)	64.8	5.62	59.5	8.48	0.001	0.0002
Tma (seconds)	1330	302	1240	554	0.40	0.038
G (dyn/cm²)	9640	444	8450	6040	0.27	0.0002
log G	9.14	0.257	8.93	0.0652	0.006	0.0002

Table 28. Mean rate of change of each of the TEG variables over the first 21 days following the SAH.

The table also shows the standard deviation of the mean rate of change and

Table 27. Summary statistics of comparison of TEG variables between the first ever and 3 month measurements in 40 SAH patients.

For all variables except R time, a tendency to hypercoagulation is found immediately following the SAH.

	Mean rate of change	Std Devn	95% confidence interval on mean	p-value from Wilcoxon signed rank test
R (secs/day)	0.38	30.75	- 6.90 to 7.65	0.41
K (secs/day)	- 3.22	12.35	- 6.14 to - 0.29	0.001
Angle (degs/day)	0.74	2.00	0.27 to 1.21	<0.0001
Ma (mm/day)	0.78	0.87	0.57 to 0.98	<0.0001
Tma (secs/day)	- 26.70	66.85	- 42.52 to - 10.8	0.0001
G (dyn/cm²/day)	524	573	388 to 659	<0.0001

Figure 23. Scatter plot of the mean R times for each of the first four weeks following SAH (1-4).

The mean R time for 3 weeks is shown at time point 3 on the x-axis.

Table 28. Mean rate of change of each of the TEG variables over the first 21 days following the SAH.

The table also shows the standard deviation of the mean rate of change and the 95% confidence interval on the mean rate of change for each parameter.

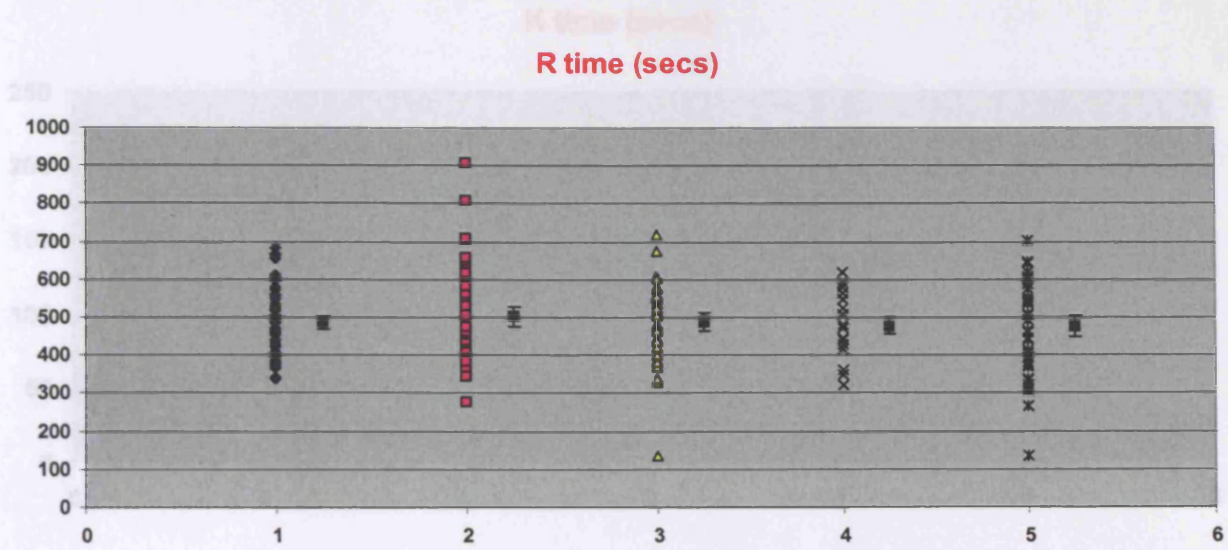


Figure 33. Scatter plot of the mean R times for each of the first four weeks following SAH (1-4).

The mean R time for 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

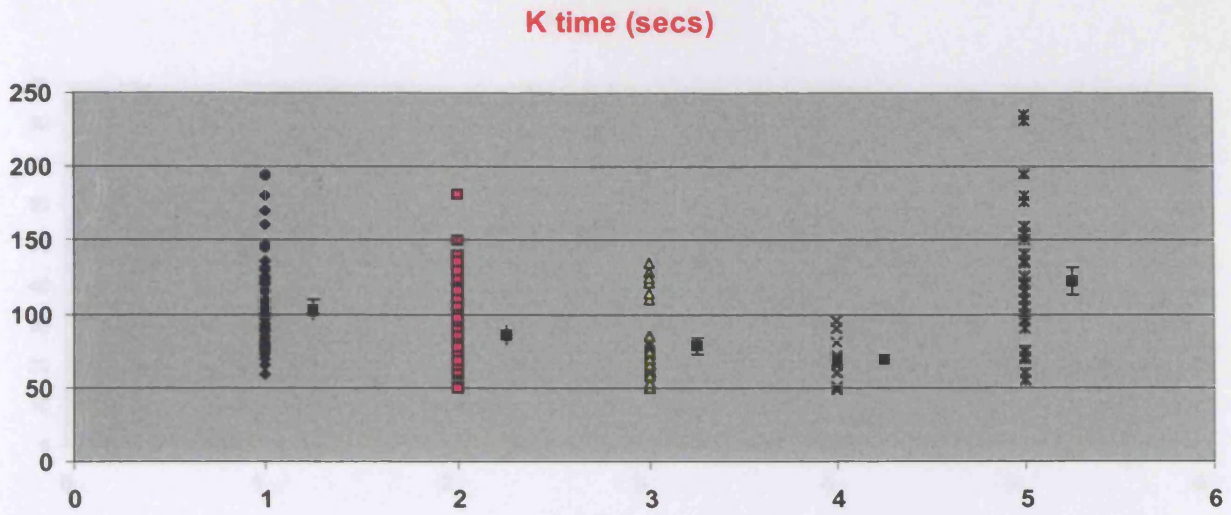


Figure 34. Scatter plot of the mean K times for each of the first four weeks following SAH (1-4).

The mean K time for 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

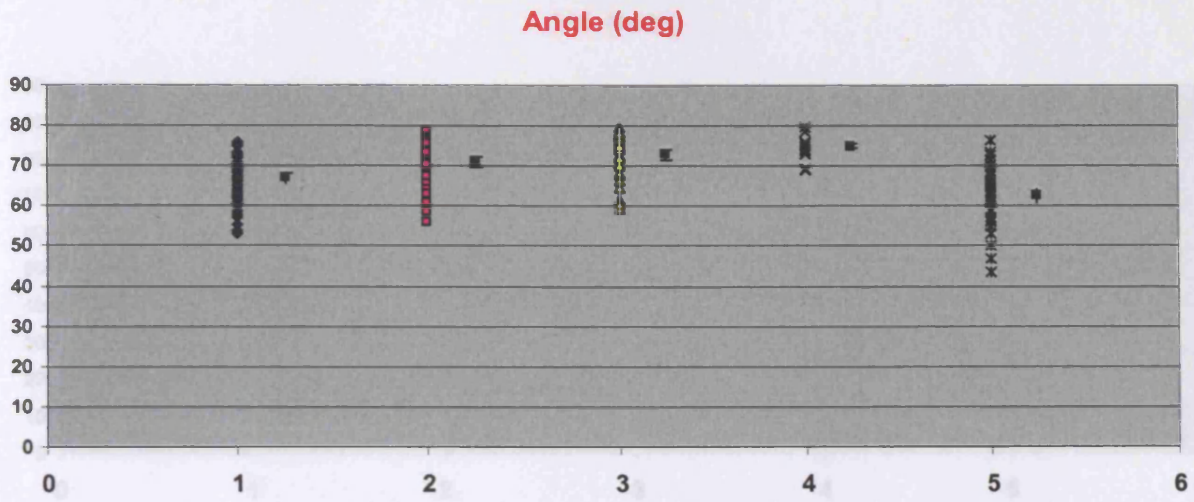


Figure 35. Scatter plot of the mean Angle for each of the first four weeks following SAH (1-4).

The mean Angle for 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

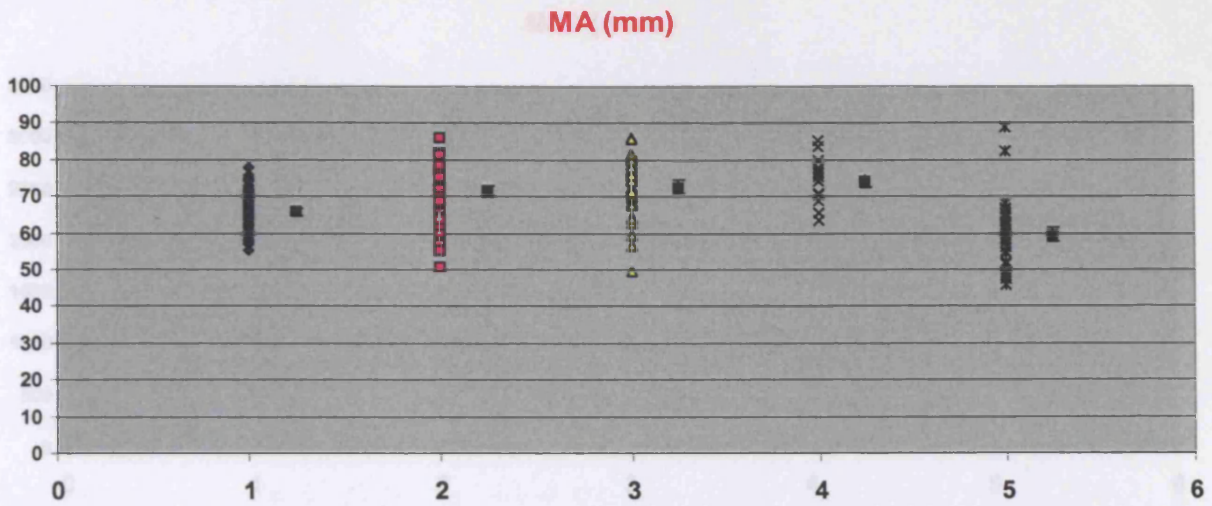


Figure 36. Scatter plot of the maximum amplitude (MA mm) for each of the first four weeks following SAH (1-4).

The mean MA for 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

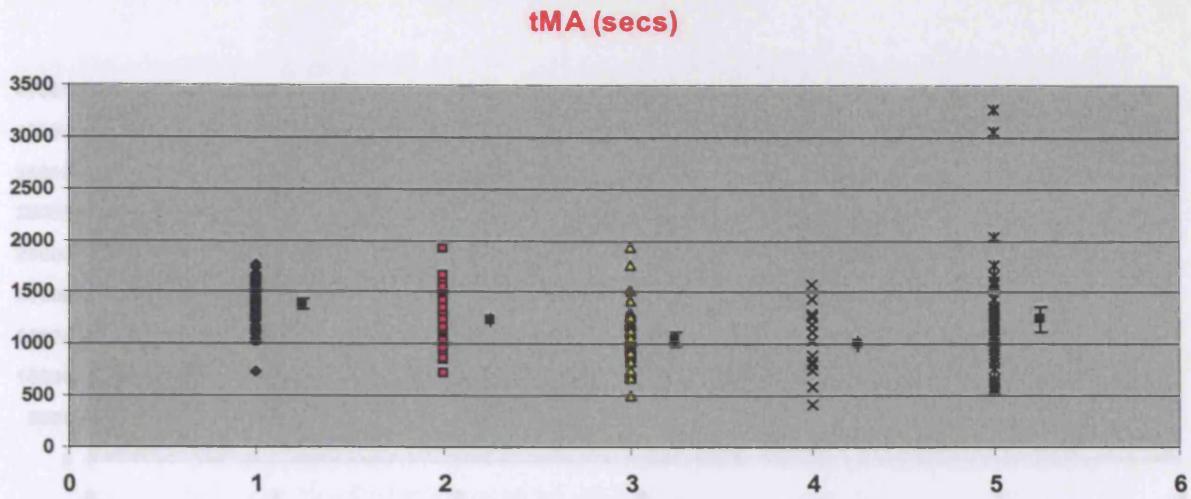


Figure 37. Scatter plot of the time to maximum amplitude (tMA secs) for each of the first four weeks following SAH (1-4).

The mean tMA for 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

G (dyn/cm²)

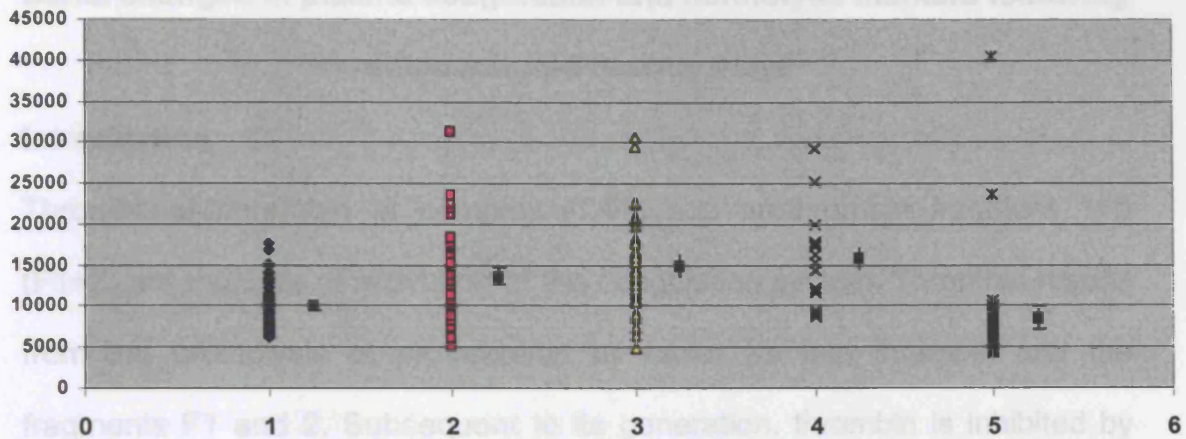


Figure 38. Scatter plot of shear elastic modulus strength G (dyn/cm²) for each of the first four weeks following SAH (1-4).

The mean G at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

Experimental study 3

Serial changes in plasma coagulation and fibrinolytic markers following subarachnoid haemorrhage

Introduction

Thrombin-antithrombin III complex (TAT) and prothrombin fragment 1+2 (F1+2) are markers of activation of the coagulation system. Thrombin results from the proteolysis of prothrombin by factor Xa into thrombin and the fragments F1 and 2. Subsequent to its generation, thrombin is inhibited by antithrombin III, with which it forms an irreversible stable complex (TAT). Though the immunochemical determination of prothrombin fragments F1+2 it is possible to quantify exactly the amount of thrombin formed.

D-dimer and plasmin-antiplasmin (PAP) complex are indicators of fibrinolysis. D-dimer rises after fibrinolysis of cross-linked fibrin. The removal of polymerised fibrin from the vascular system by proteolytic degradation (fibrinolysis) is important for maintaining the haemostatic balance. The key enzyme in the system is plasmin. An elevation of plasmin-antiplasmin complex in the plasma is caused by an excess of plasmin generation.

During early clot formation, thrombin is activated cleaving fibrinopeptides from the soluble plasma protein, fibrinogen. Molecular polymerisation occurs with formation of soluble fibrin which is then stabilised with covalent cross-linking by XIIIa activity to produce an insoluble fibrin clot. The stabilised fibrin network is immediately degraded by the fibrinolytic enzyme plasmin (fibrinolysis). Under normal physiological conditions, plasmin is rapidly neutralised by antiplasmin within the region of the clot. A variety of cross-linked fibrin degradation products are formed dependent on the extent of

fibrinolysis. The smallest fragment is the plasmin resistant species, D-dimer. Detection of D-dimer indicates the sequence of events: thrombin activation, clot formation and subsequent clot lysis.

Fibrinogen is converted to fibrin by the action of thrombin and is used to evaluate the source of fibrin clot. Fibrinogen is measured using a clot-based assay.

Methods

As for experimental study 1.

Once the TEG analysis had been started, the remaining citrated Vacutainer sample was centrifuged at 3000 rpm for 6 minutes to remove the cellular elements. The remaining supernatant was then removed and divided into 3 polypropylene tubes, taking care not to disturb the cellular debris, and contaminate the samples. The plasma was then immediately transferred to a refrigerator where it was frozen and stored at -80°C , until batch analysis of samples at a later date.

Thus, frozen plasma was obtained from 4 time periods (<48hours, 4-5, 9-10 and 15-16 days) following the ictus depending on the day of hospital admission, and on regular intervals during the hospital stay. If admission was not within one of the time periods above, a sample was taken on the day of admission to the National Hospital. In addition, a sample was taken at 3 months from the ictus.

Assay techniques

Enzyme immunoassays were used for the determination of TAT (Enzygnost® TAT micro, Dade Behring, Marburg, Germany), F1+2 (Enzygnost® F 1+2 micro, Dade Behring, Marburg, Germany), PAP

(Enzygnost® PAP micro, Dade Behring, Marburg, Germany) and D-dimer (Dimertest® GOLD STRIPWELL, AGEN, UK).

TAT, F1+2 and PAP are sandwich enzyme immunoassays. During the first incubation step the TAT (or F1+2 antigen, or PAP) present in the sample binds to the antibodies against thrombin (or F1+2, or PAP), which are attached to the surface of the microfiltration plate. Unbound constituents are then removed by washing and, in a second reaction, peroxidase-conjugated antibodies to human antithrombin III (or F1+2, or PAP) are bound to free antithrombin III (or F1+2, or PAP) determinants. The excess enzyme-conjugated antibodies are removed by washing, the bound enzyme activity is then determined. The enzymatic reaction between hydrogen peroxide and chromogen is terminated by the addition of dilute sulphuric acid. The resulting colour intensity, which is proportional to the concentration of TAT (or F1+2, or PAP), is determined photometrically.

The monoclonal antibody DD-3B6 recognises D-dimer and degradation fragments containing the D-dimer epitope. This monoclonal antibody is covalently bound to microtitre wells. When exposed to plasma or standard, this capture antibody immobilises fragments containing the D-dimer epitope. The EIA plate is then washed. Tag antibody (DD-1D2), conjugated to horseradish peroxidase is added and binds to immobilised cross-linked fibrin degradation product fragments. Colour is developed by addition of substrate, enabling cross-linked fibrin degradation product quantitation.

The Clauss fibrinogen assay was performed on a Sysmex CA-1500 analyser against an immunocoagulation reference plasma.

Statistical analysis

The first analysis involves a comparison of the 3-month measurement of TAT, P1 and 2, D-dimer, PAP and fibrinogen, with the first ever measurement following the SAH, if that measurement was within 7 days of the ictus. This analysis compared the results of 28 patients. A paired t-test and Wilcoxon signed rank tests were used to compare these measurements.

The mean rate of change of TAT, P1 and 2, D-dimer, PAP and fibrinogen was calculated over the first 21 days following SAH in each of the 67 patients. The average slopes of each variable were then compared using firstly a one-sample t-test, and then subsequently a Wilcoxon signed rank test. The latter test was chosen on statistical advice as not all the data was normally distributed, and the signed rank test is more robust in this regard.

For some of the t-tests the log of the value has been taken prior to analysis. This is because t-tests assume a normal distribution in the data, and taking logs means that the assumption of normality holds more closely. The log has no effect on the Wilcoxon signed rank test, which returns the same result, whether or not logs are used.

A p value $p < 0.05$ was taken to be significant.

Results

The results show that there were differences in the plasma assays depending on whether the measurement was taken soon after the SAH, or 3 months later (Table 29, and Figures 39 to 43). Statistically significant increases were observed in TAT complex, D-dimers, PAP complex and fibrinogen, soon after the SAH. Prothrombin fragments F1+2 did not initially rise so acutely.

The normal ranges for these assays are:

TAT complex	1.0 – 4.1 µg/l
Prothrombin fragments 1+2	0.4 – 1.10 nmol/l
D-dimers	<120 ng/ml
PAP complex	120 – 700 µg /l
Fibrinogen (Clauss)	1.5 – 4.0 g/l

TAT complex. It can be seen from Figure 39, and Tables 29, 30 and 31, that the value for TAT complex increases acutely following the SAH. It remains elevated above the normal range for the next 3 weeks. However, it does not increase progressively over this time, and remains elevated, but static. It is significantly higher in the first week following the SAH than at 3 months.

In Figures 39 to 43 the values for the fourth week are illustrated to give an impression of the wider time scale of these changes. However, because there are relatively few data points at this time only the first 21 days have been used to calculate the mean changes in each of the variables.

Prothrombin fragments 1+2. These fragments do not seem to rise as acutely, and the value in the first week is not statistically different from that at 3 months (Figure 40 and Table 29). However, by the end of the first week, it has risen above the upper limit of normal for the reference range. It continues to rise at a statistically significant rate of approximately 2% per day for the first 21 days following the SAH (Tables 30 and 31).

D-dimers. These fibrin fragments rise acutely following the haemorrhage above the normal range. They are statistically significantly elevated in the first week, compared with the samples taken at 3 months (Figure 41 and Table 29). There is no significant trend however, in its value over the first 21

days (Tables 30 and 31), and it appears to remain elevated but relatively static. Some of the patients, as with other plasma markers showed a very dramatic increase.

PAP complex. The value elevated acutely and was statistically significantly different in the first week than at 3 months (Figure 42 and Table 29). It showed a highly significant increase in the first 21 days following the ictus, increasing at an average rate of 35.4 µg //day (Tables 30 and 31).

Fibrinogen (Clauss). A similar finding was observed with fibrinogen. It rose acutely above the normal range, being higher in the first week, than at 3 months (Figure 43 and Table 29). There was a highly significant rise in the first 21 days of 0.2 g//day (Tables 30 and 31).

n=28	First measurement		3 month measurement		P value	
	Mean	SD	Mean	SD	t-test	Wilcoxon
Log TAT	1.423	0.668	1.152	0.649	0.029	0.024
Log F1+2	-0.033	0.337	-0.0825	0.278	0.44	0.633
Log D-dimer	4.483	0.742	3.815	0.77	<0.0001	<0.0001
PAP	546.36	258.11	396.28	193.71	0.0159	0.022
Fibrinogen	3.639	1.148	2.892	0.708	0.005	0.007

Table 29. The mean rate of change per day of TAT, F1+2, d-dimer, PAP

Table 29. Comparison of the first measurement taken within the first 7 days, with the 3 month value for TAT, F1+2, d-dimer, PAP and fibrinogen.

Mean and standard deviations are given, along with a p value for a paired t-test and a Wilcoxon signed rank test.

n=67	Mean	SD	95% confidence interval
Log TAT	0.0198	0.115	(-) 0.01 to 0.05
Log F1+2	0.021	0.065	0.004 to 0.038
Log D-dimer	0.026	0.117	(-) 0.05 to 0.06
PAP	35.4	51.81	21.78 to 49.02
Fibrinogen	0.195	0.21	0.14 to 0.25

Table 30. The mean rate of change per day of TAT, F1+2, d-dimer, PAP and fibrinogen, in the first 21 days following SAH.

SD = Standard deviation

SE = Standard error of the mean

P values are given using a one-sample t-test and Wilcoxon signed rank tests.

n=67	Mean	SE	p value	
			t-test	Wilcoxon
Log TAT	0.0198	0.015	0.187	0.17
Log F1+2	0.021	0.008	0.014	0.0004
Log D-dimer	0.026	0.015	0.09	0.052
PAP	35.4	6.8	<0.0001	<0.0001
Fibrinogen	0.195	0.027	<0.0001	<0.0001

Table 31. Summary statistics of the mean rate of change of each of TAT, F1+2, d-dimer, PAP and fibrinogen in the first 21 days following SAH.

SE = Standard error of the mean.

P values are given using a one-sample t-test and Wilcoxon signed rank tests.

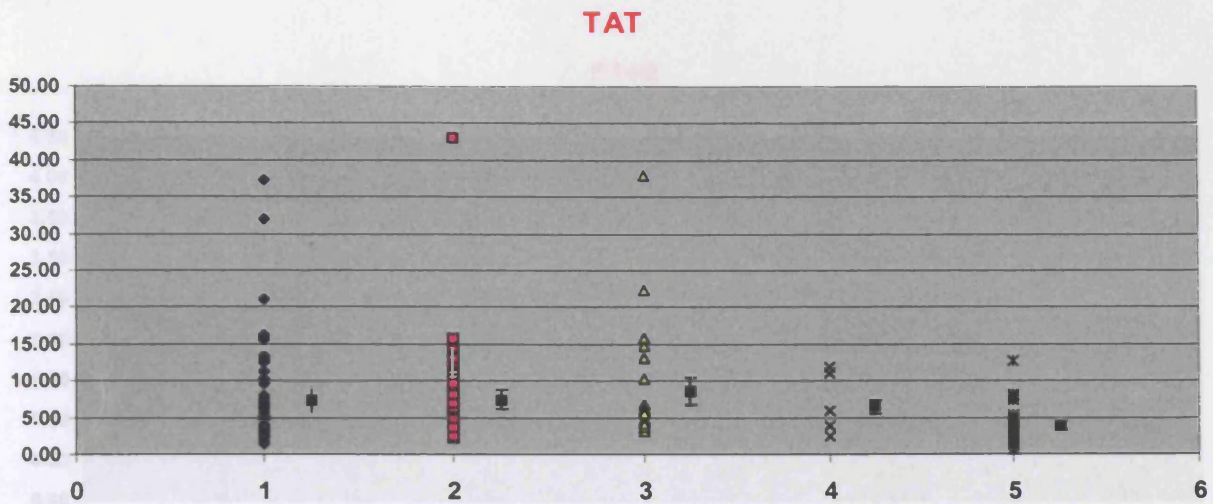


Figure 39. Scatter plot of Thrombin-Antithrombin complex (TAT) for each of the first four weeks following SAH (1-4).

The mean TAT value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

The mean and 95% confidence limits are also shown for each time period.

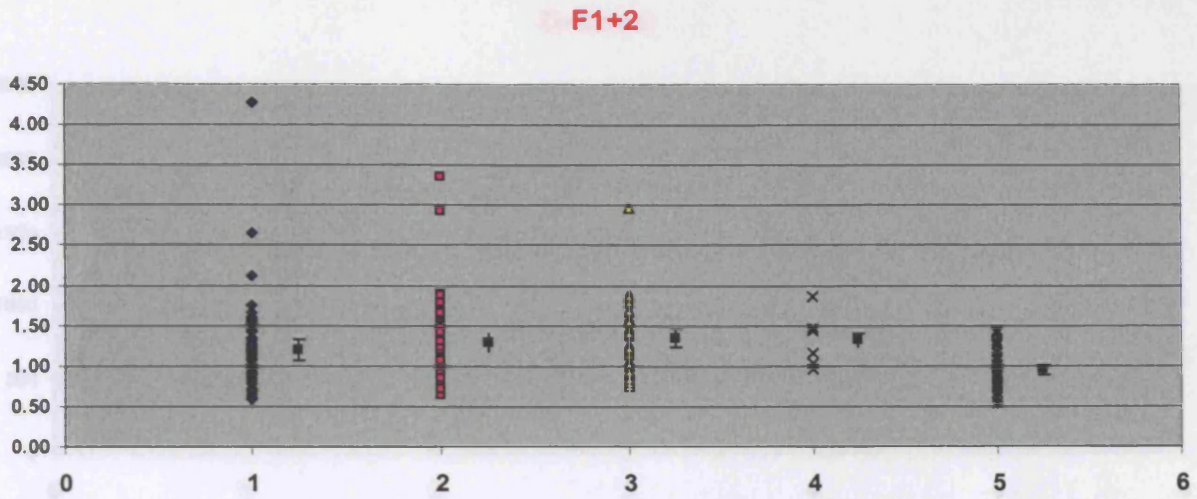


Figure 40. Scatter plot of prothrombin fragments 1 and 2 (F1+2) for each of the first four weeks following SAH (1-4).

The mean F1+2 value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

D-dimer

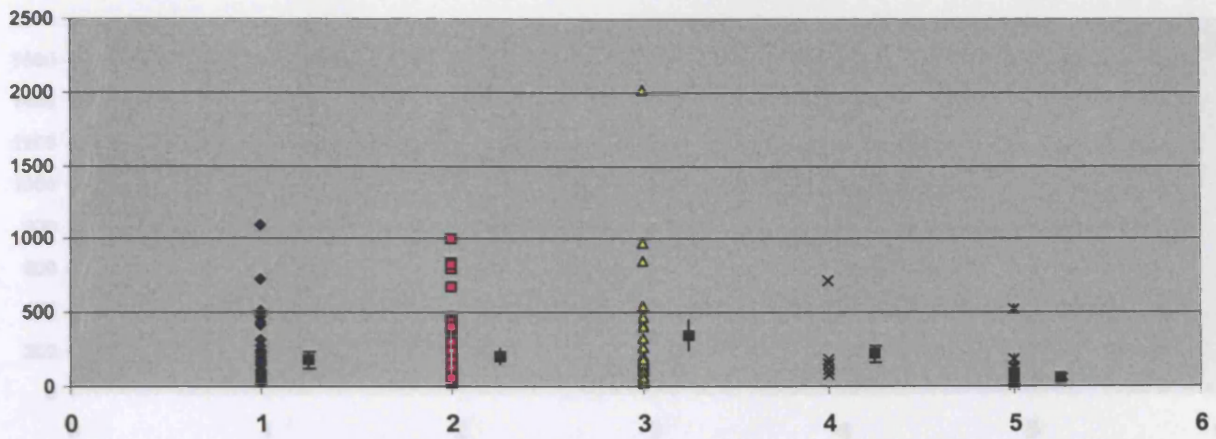


Figure 41. Scatter plot of D-dimer for each of the first four weeks following SAH (1-4).

The mean D-dimer value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

PAP

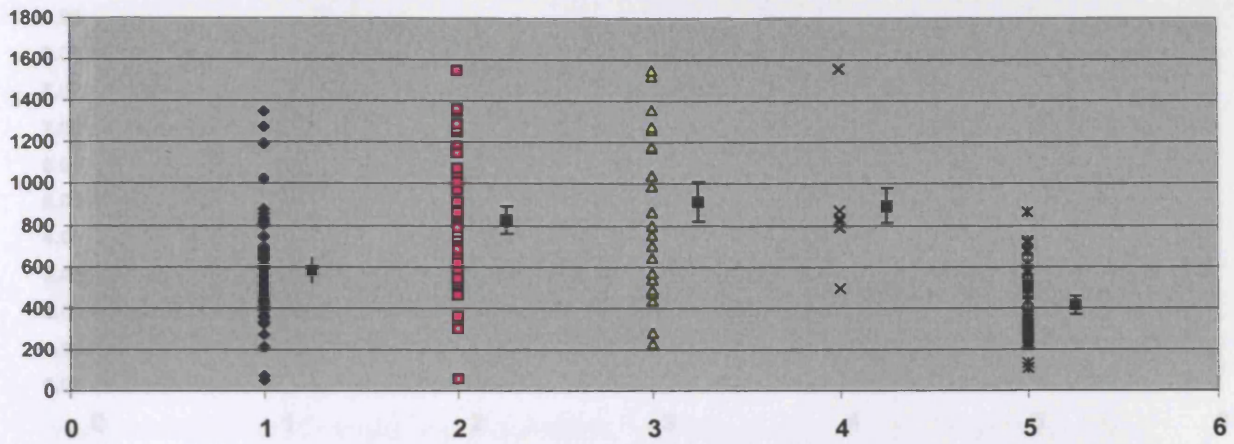


Figure 42. Scatter plot of plasmin-antiplasmin (PAP) for each of the first four weeks following SAH (1-4).

The mean PAP value at 3 months is shown at time period 5 on the x-axis. The mean and 95% confidence limits are also shown for each time period.

Fibrinogen

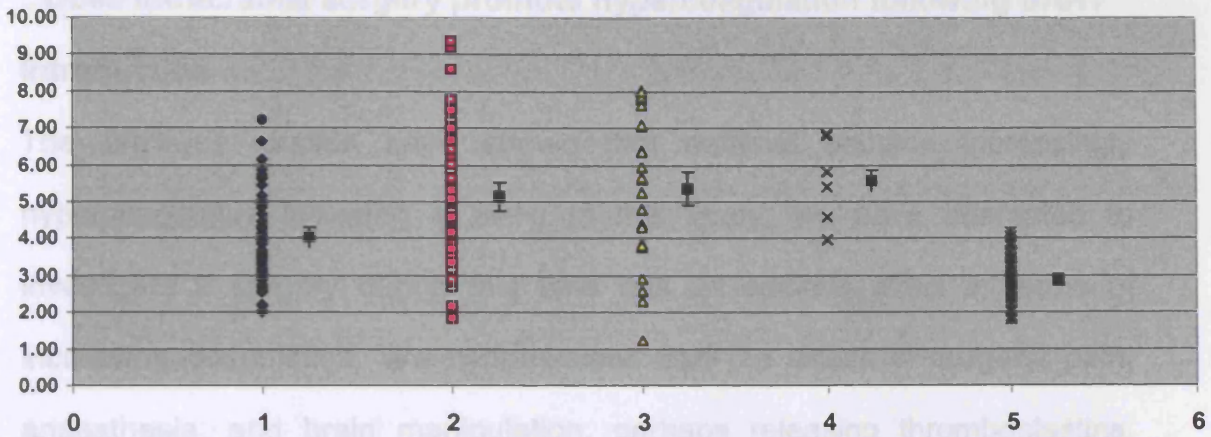


Figure 43. Scatter plot of fibrinogen for each of the first four weeks following SAH (1-4).

The mean fibrinogen value at 3 months is shown at time period 5 on the x-axis.

The mean and 95% confidence limits are also shown for each time period.

Experimental study 4

Does intracranial surgery promote hypercoagulation following SAH?

Introduction

The previous studies have shown that patients become increasingly hypercoagulable following a SAH. In this study, we have attempted to investigate if surgery during this time has an additive effect in terms of increasing coagulation. We hypothesised that the stress of surgery, pain, anaesthesia, and brain manipulation, perhaps releasing thromboplastins, may all have promoted further hypercoagulation, and that this would be evident in the rate of change of the TEG parameters following surgery.

Methods

As for experimental study 1.

Of the 67 patients recruited into this study, 43 had intracranial surgery to clip +/-wrap the aneurysm responsible for the subarachnoid bleed, and 24 patients did not receive surgery.

There is no routine policy as to when intracranial surgery should be performed on SAH patients at the National Hospital. In general, patients in good grade, with no complicating factors, following discussion with the neuroradiologists to assess if embolisation is more appropriate, will have surgery performed on the next routinely available operating list. Surgery for intracranial aneurysms rarely occurs out of normal working hours. Thus, the day on which surgery takes place following the SAH is not the same in each operated patient. The mean day on which surgery took place following the SAH in this study was 7.7 days (SD 5.12).

Of the 43 patients who had intracranial surgery, 31 had at least 2 postoperative TEG recordings distant from the time of surgery (>48 hours) but within 21 days following the SAH, and 12 had less than 2 postoperative recordings during this time period. The preoperative reading and the 2 postoperative readings were used to calculate a trend in the TEG variables for the operated patients. Thus, these trends were calculated on 31 patients. Since the operated group did not include very early times, their trends are compared with those calculated for the non operated patients between day 7 and 21 following the SAH.

Of the 24 patients who did not receive surgery, 7 were managed conservatively, 6 had suffered an angiogram negative SAH, 6 had undergone coil embolisation of their aneurysm, and 5 had died.

Details of the timing of serial TEG analysis have already been given in experimental study 1. However, 30 operative cases also had TEG analysis performed on induction of anaesthesia, 90 minutes following 'knife to skin', and 24 hours following surgery.

Statistical analysis

The first analysis performed was to compare the changes in TEG parameters around the time of surgery in 30 patients. The TEG variables were first compared on induction with 90 minutes intraoperatively, then intraoperatively with postoperatively, then finally on induction with postoperatively. Since not all of the data was normally distributed a p value has been calculated using Wilcoxon signed rank tests.

A comparison using a Mann Whitney U test was made between the very first reading performed in the non operated and operated patients. This was to

ensure that the groups were similar prior to surgery, and in particular the surgical group had not been selected from the 'better' patients.

Then a comparison of the trends i.e. the rate of change of the TEG variables per day, was made between the non operated and operated patients using a two-sample t-test with equal variance and two-sample Wilcoxon rank-sum (Mann Whitney) test. The standard deviation, standard error, and 95% confidence intervals on the mean rate of change of the TEG variables are also given.

A $p < 0.05$ was taken as significant.

Results

The first analysis involved comparing the TEG variables around the time of surgery in 30 patients (Table 32). Because of the number of significance tests caution is needed in interpreting these findings. In total 18 tests have been performed, and with a p value < 0.05 , by random chance we would expect one value to be significant. In addition some of the TEG variables are very closely related. Since no test produced a strongly significant result, there is no strong evidence of any particular change. Between the induction of anaesthesia and 24 hours later, both MA and G have increased with borderline significance. Most of the change in these variables has taken place between the intraoperative value and the postoperative test.

When comparing the first TEG profile in the non operated and operated groups (Table 33), it can be seen that there were no statistically significant differences between any of the TEG variables, suggesting that the groups were well matched prior to surgery. Indeed the means for each variable in the surgical group are on the hypercoagulable side compared with the non-

surgically treated group. Thus, in terms of their coagulation profiles, the 'best' patients have not been selected out for surgery.

A comparison of the rate of change of the TEG variables per day, up to 21 days following the SAH, between the non surgical and surgically treated patients, shows that the mean rate of change for K, Angle, MA and G is toward the more hypercoagulable in the surgically treated group. However, this does not reach statistical significance for any variable (Tables 34 and 35 and Figures 44 to 46).

	Immediately prior to operation		During operation		Within one day post operation		p-value for comparing prior and during	p-value for comparing prior and after	p-value for comparing during and after
	Mean	SD	Mean	SD	Mean	SD	Wilcoxon signed rank test	Wilcoxon signed rank test	Wilcoxon signed rank test
R (seconds)	437	125	414	108	487	138	0.50	0.044	0.032
K (seconds)	83.0	31.1	75.3	3.64	85.4	29.6	0.21	0.18	0.099
Angle (degrees)	71.4	6.17	71.8	7.07	71.0	5.10	0.54	0.41	0.22
MA (mm)	67.9	6.10	69.1	5.97	70.8	6.10	0.19	0.004	0.088
TMA (seconds)	1220	351	1375	194	1320	249	0.019	0.16	0.24
G (dyn/cm ²)	11300	4210	11800	3670	13000	4280	0.27	0.012	0.084
log G	9.28	0.303	9.34	0.29	9.42	0.307	0.27	0.012	0.084

Table 31. Comparison of the five TEG profiles performed in the non-surgically and surgically treated patients.

SD = Standard deviation

Table 32. The changes in TEG variables (R, K, Angle, MA and G) around the time of surgery.

SD = Standard deviation

Prior = On induction.

During = 90 minutes following 'knife to skin'.

After = 24 hours following surgery.

	Non-Surgical Patients N=24		Surgical Patients N=43		Mann-Whitney U test
	Mean	SD	Mean	SD	p-value
R (secs)	503	95	478	103	0.22
K (secs)	112	35.6	101	30.9	0.18
Angle (degs)	65	7.19	67.3	5.89	0.15
MA (mm)	63.5	6.03	65.3	5.66	0.43
TMA (secs)	1470	300	1300	273	0.37
G (dyn/cm ²)	9150	2930	9830	2680	0.43

Table 33. Comparison of the first TEG profile performed in the non-surgically and surgically treated patients.

SD = Standard deviation

n=67	Non-operated			Operated		
	Mean	SE	95% CI	Mean	SE	95% CI
R	515.5	22.5	468.4 - 562.6	461.5	20.3	420.4 - 502.6
K	93.7	6.86	79.3 - 108	85.4	5.68	73.9 - 96.9
Angle	69.3	1.4	66.4 - 72.2	71	1.06	68.8 - 73.1
MA	68.6	1.63	65.1 - 72	69.8	1.05	67.7 - 71.9
tMA	1202.5	73.5	1048.7 - 1356.3	1289.9	56.8	1174.8 - 1405
G	11928	1088.7	9649.2 - 14206.7	12497	777.8	10921.3 - 14073.1

Table 35. A comparison of the rate of change of each TEG variable per day following surgery, with those patients who did not have surgery.

Table 34. Comparison of the mean, standard error (SE), and 95% confidence intervals (CI), for each TEG variable, in the non-operated and operated patients.

	Trend in Non-operative Patients n=20		Trend in Operative patients n=31		Mann-Whittney U test	two-sample t test
	Mean	SD	Mean	SD	p-value	p-value
R (sec/day)	-3.36	19.9	2.56	45.6	0.24	0.1
K (sec/day)	-1.13	6.03	-2.68	17.2	0.63	0.38
Angle (deg/day)	0.288	1.2	0.689	2.72	0.69	0.38
MA (mm/day)	0.38	1.41	0.565	1.146	0.63	0.51
TMA (sec/day)	-28.2	56.7	-34.9	81.8	0.66	0.36
G (dyn/cm2/day)	238	839	483	894	0.32	0.67

Table 35. A comparison of the rate of change of each TEG variable per day following surgery, with those patients who did not have surgery, for the first 21 days following the SAH.

SD = Standard deviation.

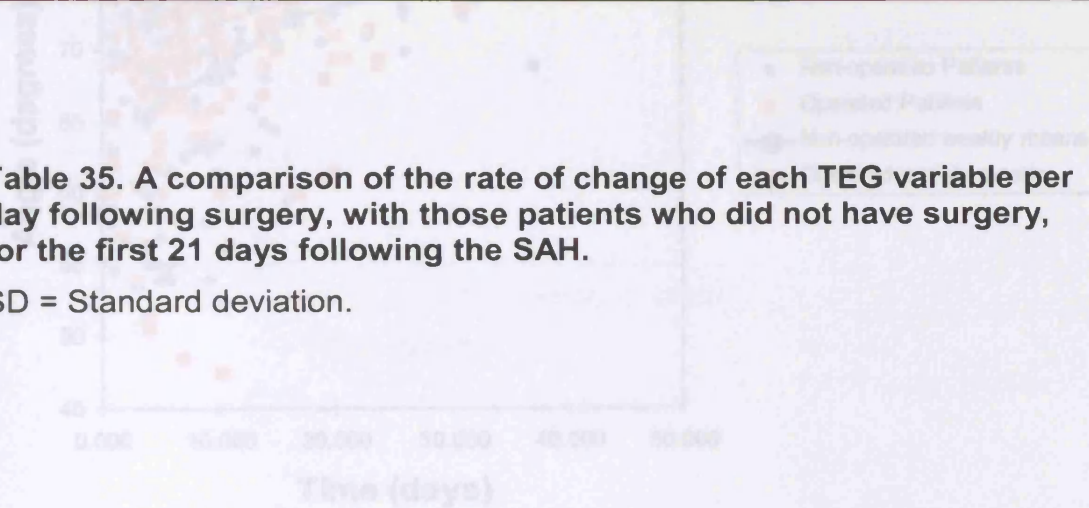


Figure 44. Scatter plot of TEG Angle values for operated and non-operated patients

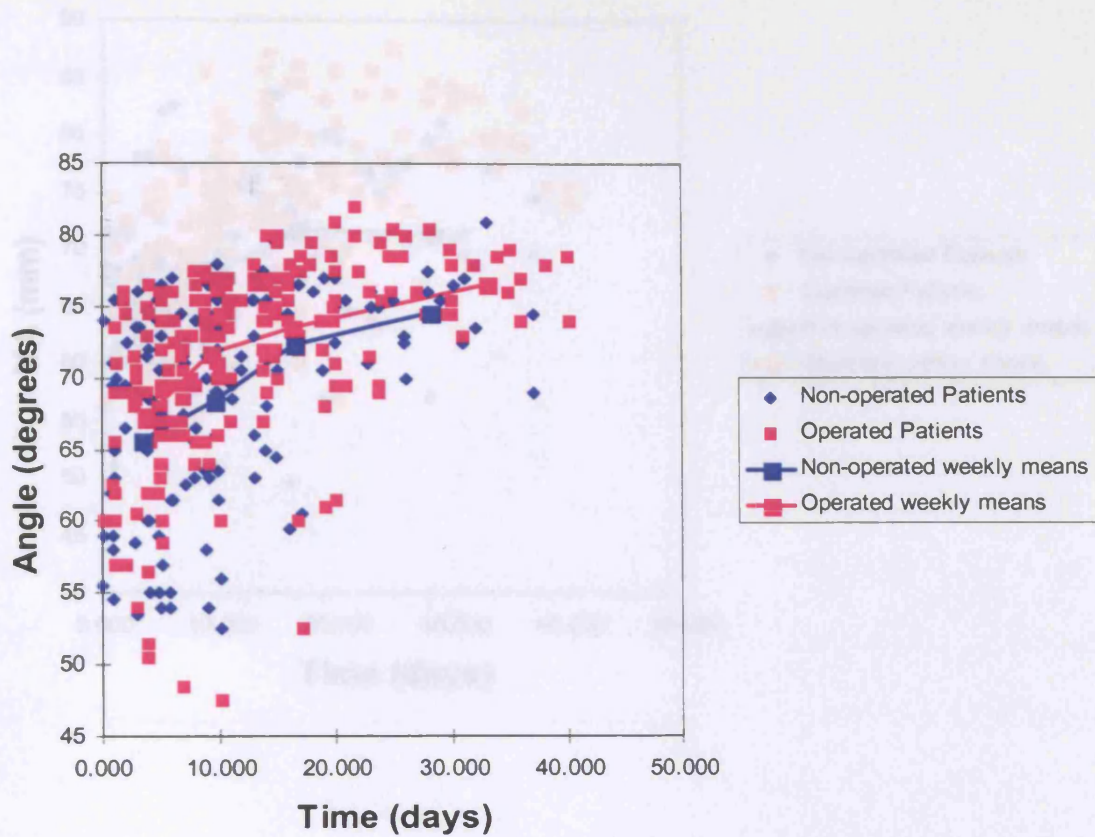


Figure 45. Scatter plot of TEG Angle values for operated and non-operated patients

Figure 44. Scatter plot of TEG Angle values for operated and non-operated patients

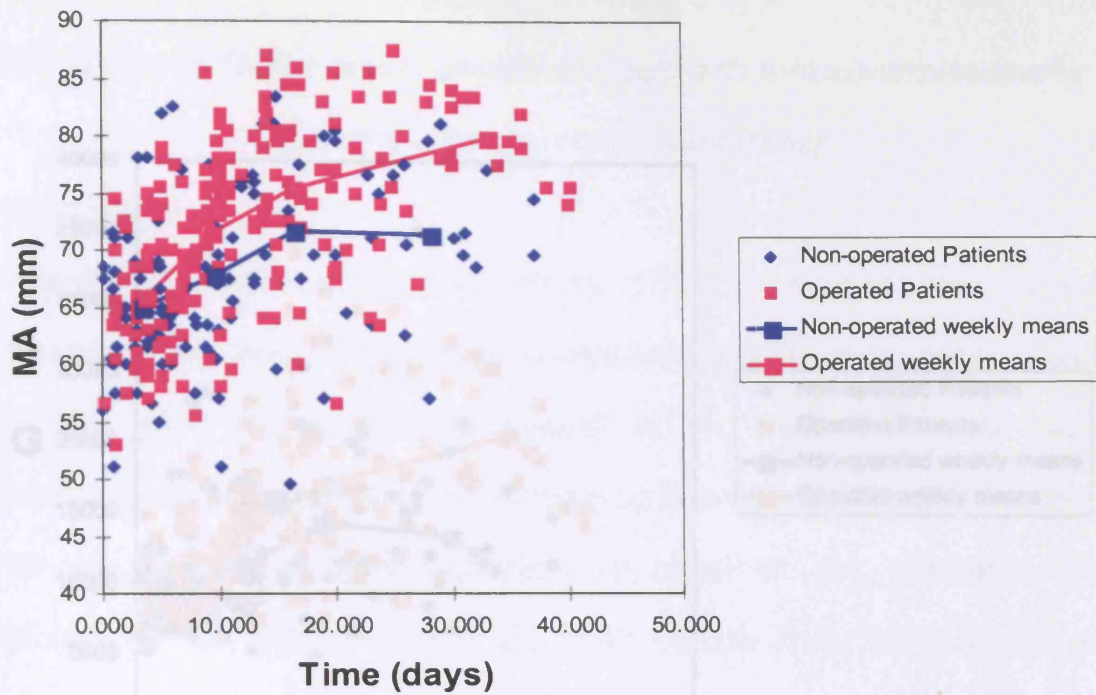


Figure 45. Scatter plot of TEG MA values for operated and non-operated patients

Correlation of Fisher grade, gender and age with thromboelastography following subarachnoid haemorrhage

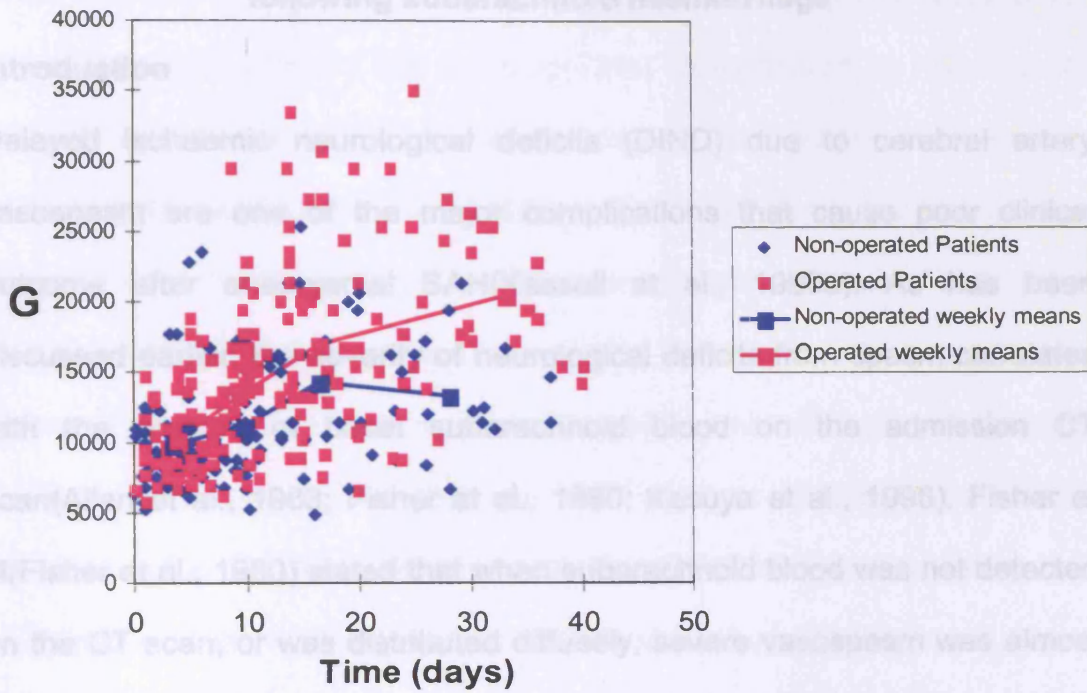


Figure 46. Scatter plot of TEG G values for operated and non-operated patients

Experimental study 5

Correlation of Fisher grade, gender and age with thromboelastography following subarachnoid haemorrhage

Introduction

Delayed ischaemic neurological deficits (DIND) due to cerebral artery vasospasm are one of the major complications that cause poor clinical outcome after aneurysmal SAH(Kassell et al., 1990a). As has been discussed earlier, the severity of neurological deficits from spasm correlates with the amount of basal subarachnoid blood on the admission CT scan(Allen et al., 1983; Fisher et al., 1980; Kasuya et al., 1998). Fisher et al(Fisher et al., 1980) stated that when subarachnoid blood was not detected on the CT scan, or was distributed diffusely, severe vasospasm was almost never encountered. However, in the presence of subarachnoid blood clots larger than 5 x 3 mm, of layers of blood 1mm or more thick in the fissures and vertical cisterns, severe spasm followed almost invariably. They noted an almost exact correspondence between the site of major subarachnoid blood clot and the location of severe spasm. They suggested that blood clots are especially spasmogenic when they lie along or in contact with the major arteries of the circle of Willis, particularly the proximal 9 to 10cm of the anterior and middle cerebral arteries.

Fisher et al(Fisher et al., 1980) classified the subarachnoid blood found on CT imaging into four grades: Grade 1, no blood detected; Grade 2, a diffuse deposition or thin layer, with all vertical layers less than 1 mm thick; Grade 3, localised clots and/or vertical layers of blood greater than 1mm thick; Grade

4, diffuse or no subarachnoid blood, but with intracerebral or intraventricular clots (Appendix VIII).

Jarus-Dziedzic et al (Jarus-Dziedzic et al., 2000) concluded in a study of 127 aneurysmal SAH patients that the blood load as measured by Fisher grade was significantly correlated with cerebral blood flow velocity measurements using transcranial doppler. These authors found that during the first 21 days following SAH the highest flow velocities were observed in the Fisher grade III and II patients. Lower values were found in patients with massive SAH (group IV) and the lowest flow velocities were recorded in group I patients, that is those without SAH on the original CT scan. In addition the number of days for which the mean flow velocity in the middle cerebral artery was $>120\text{cm/s}$ was statically correlated with blood load on CT imaging (I, II and III). In all patients the greatest difference in mean flow velocity measurements were observed between the 9th and 14th day following SAH. The authors suggest that the apparent failure to demonstrate consistent trend with regard to the grade IV patients could be explained by raised ICP and thus decreased cerebral blood flow in these patients. Similar findings of increased ischaemic deficit and increased doppler flow velocities were also observed by Grosset et al (Grosset et al., 1994) in relation to increased blood load. Once again however, Fisher grade IV patients were not in agreement with this trend. The authors suggest that this may be explained by the fact that intracerebral and intraventricular blood can be present without significant volumes of subarachnoid blood.

Fujii et al found no significant relationship between the severity of subarachnoid clot and the levels of fibrinogen, D-Dimer, and thrombin-

antithrombin complex following SAH(Fujii et al., 1997). Though in a previous study(Fujii et al., 1995), they had suggested that the level of TAT, D-dimer and PAP complexes, were all increased with the severity of neurological status, Fisher grade, and severity of clinical outcome.

However, because heavy CT blood load also correlates with other prognostic indicators such as, poor condition on admission, coexistent hypertension, age and surgery, the causative relationship between blood load and overall clinical outcome is complex(Grosset et al., 1994).

In this study we investigate whether haemostatic parameters (measured using TEG), reflect the severity of CT findings of SAH (measured using Fisher grade). If this is the case, then TEG may be a useful clinical tool in predicting prognosis, and monitoring treatment strategies.

In addition, after observing differences in TEG parameters in a control population with respect to gender, but not age (control study 2), we have investigated the effect of these variables on the haemostatic response following SAH.

Methods

As for experimental study 1.

Four of the patients in clinical study 1 did not have a CT scan performed. Thus, this is a prospective observational study in 63 consecutive patients admitted to the NHNN with a primary diagnosis of SAH confirmed by CT imaging.

There were 22 males (mean age 50.5 years, SD 15.9) and 41 females (mean age 53.4 years, SD 13.8). The cause of SAH was a ruptured cerebral aneurysm in 54 patients, an arteriovenous malformation in 2, the angiogram was negative in 5, and no angiogram was performed in 2 patients.

Fisher grading of the CT scans was undertaken by myself and independently by a consultant neurosurgeon (Mr. Neil D. Kitchen). All admission CT brain scans were analysed.

There were 3 Fisher grade 1 patients, 22 grade 2, 16 grade 3 and 22 grade 4. The first regression analysis showed that there was no difference in TEG variables when divided into 4 groups by Fisher grade. However, because there were only 3 patients in Fisher grade 1, doubts were raised as to the significance of these findings. Therefore, it was decided to group patients before the regression analysis.

Group 1 'Good' grade (Fisher 1 and 2)

Group 2 'Poor' grade (Fisher 3 and 4)

The mean value of the TEG variables R, K, Angle, MA and G were taken for the first 14 days following the SAH. These were compared in turn with age, gender and group, to establish if any correlation existed.

Statistical analysis

To compare for differences, the mean TEG value in the first 14 days was regressed in turn for age, gender and group.

A p-value of <0.05 was taken as significant.

Results

The results demonstrate that for the TEG parameter R, there was no statistically significant difference due to group ($F(3,62)=0.88;p>0.05$), age ($F(3,62)=0.88;p>0.05$) or gender ($F(3,62)=0.88;p>0.05$) (Tables 36, 41 and 42, Figure 47). The regression analysis of K also showed a non-significant result for group ($F(3,62)=5.07;p>0.05$), and age ($F(3,62)=5.07;p>0.05$) however, the analysis showed a significant effect of gender ($F(3,62)=5.07;p<0.01$) (Tables 37, 41 and 43, and Figure 48). Regression analysis of Angle also showed that there was no statistically significant difference due to group ($F(3,62)=5.84;p>0.05$) or age ($F(3,62)=5.84;p>0.05$) but again gender effects were found ($F(3,62)=5.84;p<0.01$) (Tables 38 and 41).

Regression analysis of TEG parameters MA and G also showed no statistical difference due to group (MA: $F(3,62)=3.71;p>0.05$ and G: $F(3,62)=3.65;p>0.05$), and age for G: $F(3,62)=3.65;p>0.05$). There was a significant effect for age identified in MA: $F(3,62)=3.71;p<0.05$. However, both MA and G showed a statistical difference due to gender (MA: $F(3,62)=3.71;p<0.05$ and G: $F(3,62)=3.65;p<0.05$) (Tables 39, 40, 41 and 44, and Figure 49).

Thus, in summary no association was established between any TEG variable and Fisher grade as represented by group. Only MA showed an association

with age, and this was only weakly significant. However, the TEG variables K, Angle MA and G in the first 14 days following a SAH were significantly associated with gender. In each case the mean value for the female variable was hypercoagulable compared to the male value (Table 41, and Figures 48 and 49).

Regression Statistics	
Multiple R	0.21
R Square	0.04
Adjusted R Square	-0.01
Standard Error	74.63
Observations	63

ANOVA

	df	SS	MS	F	Significance F
Regression	3	14739.15	4913.05	0.88	0.46
Residual	59	328576.77	5569.09		
Total	62	343315.92			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	444.12	52.52	8.46	9.28E-12	339.04	549.21
age	0.08	0.66	0.12	0.90	-1.24	1.40
Group	-2.29	19.44	-0.12	0.91	-41.19	36.62
sex	32.44	19.98	1.62	0.11	-7.53	72.41

Table 36 Analysis of the effects of group, gender and age on the TEG parameter R following SAH.

Using $p < 0.05$ to determine significance, regression analysis of R for effects of group, gender and age revealed that neither group, gender or age produced a significant effect on R ($p > 0.05$).

Regression Statistics	
Multiple R	0.45
R Square	0.20
Adjusted R Square	0.16
Standard Error	23.43
Observations	63

ANOVA

	df	SS	MS	F	Significance F
Regression	3	8351.08	2783.69	5.07	0.003
Residual	59	32392.61	549.03		
Total	62	40743.69			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	80.51	16.49	4.88	8.347E-06	47.51	113.50
age	-0.37	0.21	-1.78	0.08	-0.78	0.05
Group	5.51	6.11	0.90	0.37	-6.71	17.72
sex	19.29	6.27	3.08	0.003	6.74	31.84

Table 37. Analysis of the effects of group, gender and age on the TEG parameter K following SAH.

Using $p < 0.05$ to determine significance, regression analysis of K for effects of group, gender and age revealed that neither group nor age produced a significant effect on K ($p > 0.05$).

There was a significant effect of gender on K ($p < 0.01$).

Regression Statistics	
Multiple R	0.48
R Square	0.23
Adjusted R Square	0.19
Standard Error	4.62
Observations	63

ANOVA

	df	SS	MS	F	Significance F
Regression	3	373.79	124.60	5.85	0.001
Residual	59	1256.83	21.30		
Total	62	1630.62			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	71.59	3.25	22.04	3.624E-30	65.09	78.09
age	0.08	0.04	1.87	0.07	-0.01	0.16
Group	-0.88	1.20	-0.73	0.47	-3.29	1.53
sex	-4.19	1.24	-3.39	0.001	-6.67	-1.72

Table 38. Analysis of the effects of group, gender and age on the TEG parameter Angle following SAH.

Using $p < 0.05$ to determine significance, regression analysis of Angle for effects of group, gender and age revealed that neither group nor age produced a significant effect on Angle ($p > 0.05$).

There was a significant effect of gender on Angle ($p < 0.01$).

Regression Statistics	
Multiple R	0.40
R Square	0.16
Adjusted R Square	0.12
Standard Error	5.70
Observations	63

ANOVA

	df	SS	MS	F	Significance F
Regression	3	361.72	120.57	3.71	0.02
Residual	59	1915.77	32.47		
Total	62	2277.49			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	67.15	4.01	16.75	4.345E-24	59.13	75.18
age	0.11	0.05	2.18	0.03	0.01	0.21
Group	-0.45	1.48	-0.30	0.76	-3.42	2.52
sex	-3.44	1.53	-2.25	0.03	-6.49	-0.39

Table 39. Analysis of the effects of group, gender and age on the TEG parameter MA following SAH.

Using $p < 0.05$ to determine significance, regression analysis of MA for effects of group, gender and age revealed that group had no significant effect on ($p > 0.05$).

There was a significant effect of gender and age on MA ($p < 0.05$).

Regression Statistics	
Multiple R	0.34
R Square	0.16
Adjusted R Square	0.11
Standard Error	3250.91
Observations	63

ANOVA

	df	SS	MS	F	Significance F
Regression	3	115631576	38543859	3.65	0.02
Residual	59	623538279	10568445		
Total	62	739169856			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	11637.73	2287.75	5.08	3.966E-06	7059.96	16215.5
age	54.433114	28.75	1.89	0.06	-3.09	111.95
Group	-105.93	847.03	-0.13	0.90	-1800.85	1588.98
sex	-2159.44	870.16	-2.48	0.02	-3900.64	-418.25

Table 40. Analysis of the effects of group, gender and age on the TEG parameter G following SAH.

Using $p < 0.05$ to determine significance, regression analysis of G for effects of group, gender and age revealed that neither group nor age produced a significant effect on G ($p > 0.05$).

There was a significant effect of gender on G ($p < 0.01$).

n=67	Female		Male		F	P-value
	Mean	SD	Mean	SD		
R	477.3	71.6	509.2	76.7	0.88	0.109
K	58.7	21.9	109.7	26.9	5.07	0.003
Angle	70.1	4.5	65.6	5.1	5.85	0.001
MA	68.9	6.2	65.1	5	3.71	0.03
G	12219	3723.3	9889.6	2256.9	3.65	0.02

Table 41. Summary of the regression analysis for gender for the TEG variables taken within the first 14 days following SAH.

SD = Standard Deviation.

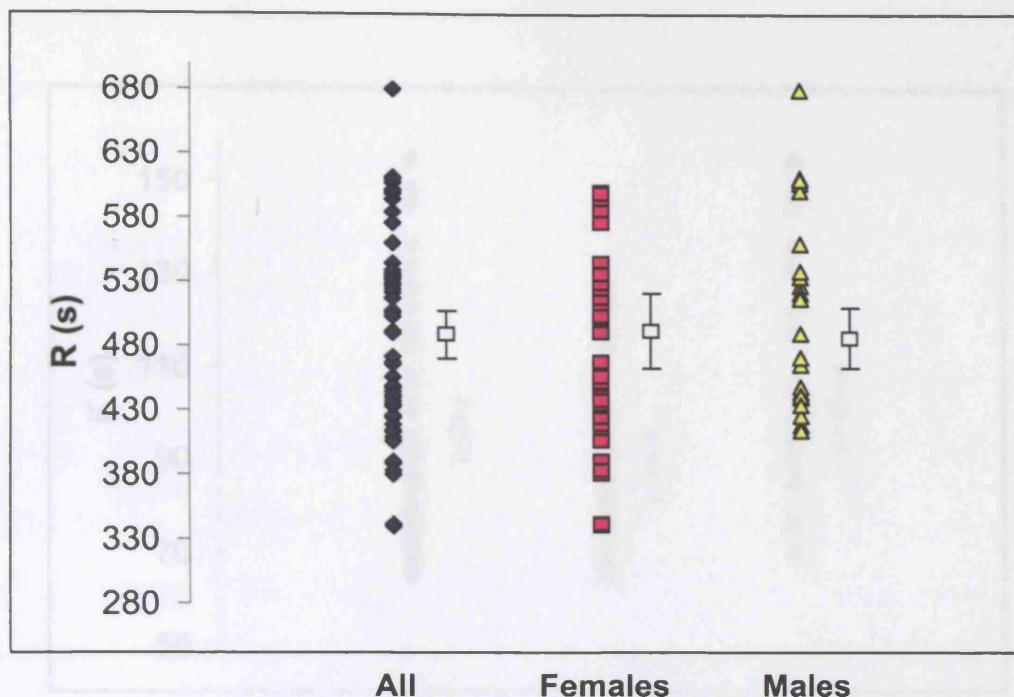
	Mean	Standard Deviation	Confidence Interval
All	489.47	74.41	19.36
Females	477.32	71.52	21.97
Males	509.25	76.71	32.05

Figure 47. Scatter diagram of R for males and females following SAH.

There was no significant difference between males and females for TEG parameter R ($p > 0.05$).

Table 42. Summary table of R for males and females following SAH.

The table shows the mean, SD and CI for All subjects, female subjects and male subjects for TEG variable R.



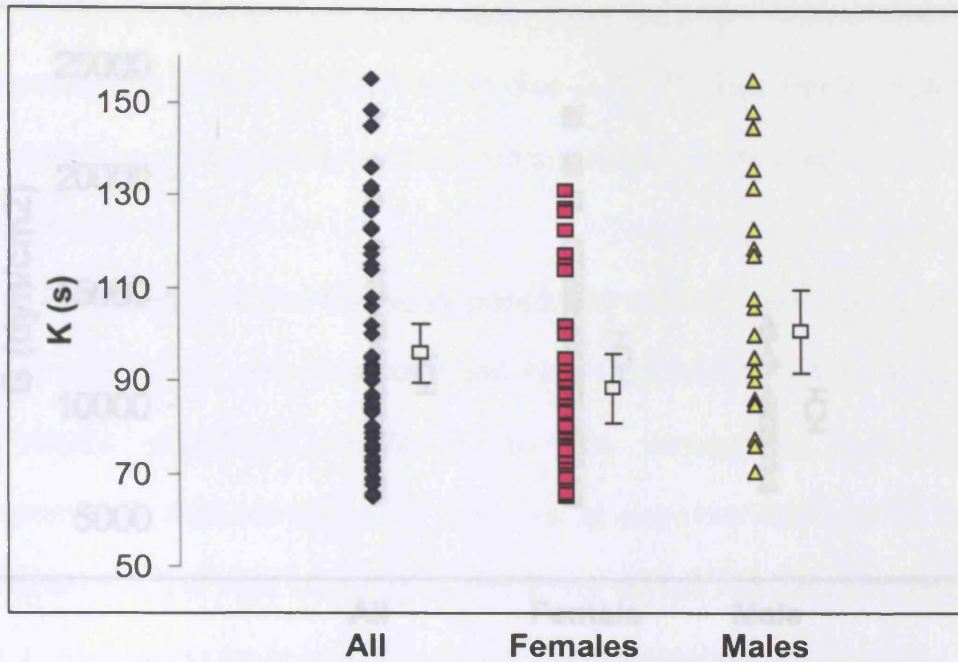
	Mean	Standard Deviation	Confidence Interval
All	488.47	74.41	18.38
Females	477.32	71.62	21.92
Males	509.25	76.71	32.05

Figure 47. Scatter diagram of R for males and females following SAH.

There was no significant difference between males and females for TEG parameter R ($p > 0.05$).

Table 42. Summary table of R for males and females following SAH.

The table shows the mean, SD and CI for All subjects, female subjects and male subjects for TEG variable R.



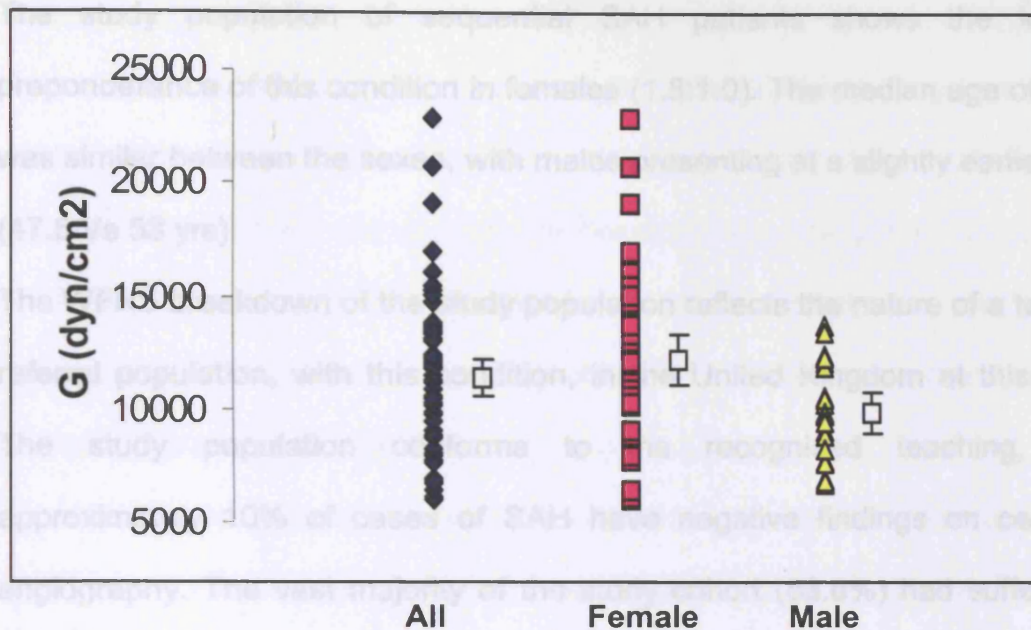
	Mean	Standard Deviation	Confidence Interval
All	96.07	25.64	6.33
Females	88.73	21.94	6.72
Males	109.75	26.87	11.23

Figure 48. Scatter diagram of K for males and females following SAH.

There was a significant difference between males and females for TEG parameter K ($p < 0.01$).

Table 43. Summary table of K for males and females following SAH.

The table shows the mean, SD and CI for All subjects, female subjects and male subjects for TEG variable K.



	Mean	Standard Deviation	Confidence Interval
All	11405.63	3452.84	852.62
Females	12219.13	3723.25	1139.67
Males	9889.56	2256.90	943.08

Figure 49. Scatter diagram of G for males and females following SAH.

There was a significant difference between males and females for TEG parameter G ($p < 0.05$).

Table 44. Summary table of G for males and females following SAH.

The table shows the mean, SD and CI for All subjects, female subjects and male subjects for TEG variable G.

Discussion

The study population of sequential SAH patients shows the known preponderance of this condition in females (1.8:1.0). The median age of SAH was similar between the sexes, with males presenting at a slightly earlier age (47.5 Vs 53 yrs).

The WFNS breakdown of the study population reflects the nature of a tertiary referral population, with this condition, in the United Kingdom at this time. The study population conforms to the recognised teaching, that approximately 10% of cases of SAH have negative findings on cerebral angiography. The vast majority of the study cohort (83.6%) had suffered a SAH secondary to the rupture of an intracranial cerebral aneurysm.

In the studies, I have taken serial measurements of haemostatic and haematological variables around the time period when it is recognised that, the symptoms of vasospasm present most frequently (5-12 days following SAH). Because of wide variations of haematological variables in control populations, I have taken a distant sample (3 months), in the studied patients. In this way each patient is effectively acting as his or her own control. The assumption being that if the patient was well enough to attend the outpatient department at this time, any acute changes in haematological variables as a result of the SAH, should have returned, or be returning to normal for that patient.

Unfortunately, because of delays in presentation, diagnosis, and transfer of patients, it was not always possible to get a sample within 48 hours of the ictus. In addition, not all patients have presented to the outpatient clinic, for a 3-month sample. This can be explained by death, or severe disability in

some of the study cohort, and some patients chose for geographical reasons, to be followed up at other units.

By using a standard method of sample collection, handling, and TEG analysis throughout the control and clinical studies, I have reduced the chances of the observed changes in haemostasis, being due to technical error. My own and others studies have demonstrated, that the results obtained by TEG analysis are comparable with standard venous and arterial sampling(Spiess et al., 1987).

By excluding patients with known coagulopathies, I have attempted to ensure that I am observing the systemic changes as a result of the SAH and its treatment, and not changes related to an intrinsic problem with haemostasis specific to particular individuals. SAH patients were not given heparin, aspirin, other non-steroidal anti-inflammatory medications, or corticosteroids, which may have interfered with the haemostatic process during the study.

The TEG data shows evidence of increased coagulation following the ictus, when compared to the value 3 months later in the same patient. All of the TEG variables show a hypercoagulable change (K, Angle, MA, tMA and G), except R. This is an interesting finding, as the R time is said to provide information analogous to that of the routine coagulation tests PT and APPT, as it is the time until the first fibrin strands start to form. This suggests that these differences would not have been found using such tests. Thus, the data provides evidence of an increased rate of fibrin build up and cross-linking (K and Angle), and increased strength and stability of the final clot (MA and G), following SAH. The final strength of the clot is particularly sensitive to platelet number and function, and fibrinogen concentration.

In addition, the TEG data demonstrates that there is ever increasing hypercoagulability using these markers, over the first 21 days following SAH. This data is highly significant, and once again the only variable not statistically changed over time, is the R time. This suggests that the haemostatic process takes a similar time to start to form fibrin in all patients, however, once it is initiated, it forms more quickly, with more cross-linking, and platelet-fibrinogen binding, during the first 21 days following SAH.

TEG has never previously been used to investigate coagulation in SAH. Previous studies have suggested that comprehensive haemostatic assays may predict the severity of aneurysmal SAH and the risk of DIND(Fujii et al., 1997). It has never been previously suggested that patients become increasingly hypercoagulable in the days following a SAH. A state of increased thrombogenicity may increase the risk of cerebral ischaemia and infarction, by increasing the incidence of microembolic events.

It remains to be established at what point following a SAH, the trend in coagulation changes, and procoagulant and fibrinolytic markers start to fall. Certainly they have fallen by 3 months. It can be seen that although there is an increase in TEG coagulation markers each week, the trends appear to show a reduction in this increase week by week, up to the fourth week.

The plasma assays show similar findings to the TEG data. There is evidence of initiation of coagulation and fibrinolysis following the ictus. The markers also show that coagulation and fibrinolysis appears to be increasingly triggered each day, for the first 21 days following a SAH. Previously it has been suggested that these changes show no overall change in the balance between coagulation and fibrinolysis. An alternative explanation is that

increased PAP and D-dimers are merely a reflection that much more fibrin is being generated. When taken in conjunction with the TEG data, it appears that the overall balance has swung in favour of a hypercoagulable and procoagulant response following SAH.

The fact that TAT does not appear to increase over time at the same rate as the prothrombin fragments F1+2, is probably explained by variability in the TAT assay. Taken in conjunction with other tests, there appears overwhelming evidence of increased thrombin generation in the weeks following a SAH. A similar trend in TAT has been observed by other authors(Fujii et al., 1997; Itoyama et al., 1994).

The results of the fibrinolytic markers also provide evidence of the balance occurring between the opposing systems. The PAP increases steadily over time, but the D-dimer value, although elevated does not increase at the same rate. This suggests increased fibrinolytic potential, but not increased fibrinolysis. There are increasing amounts of plasmin generated, perhaps in response to the increased thrombin formation. However, one can postulate that this is quickly mopped up by circulating antiplasmin and inactivated. Thus, there is no increase in the concentration of D-dimers, because the inactivated plasmin is unable to break down fibrin. Thus, the increased plasmin generation is controlled.

Similar trends have been reported previously for D-dimers following SAH(Fujii et al., 1995; Fujii et al., 1997; Nina et al., 2001). However, previous studies have reported that the PAP level is highest in the first week, and then falls very slowly subsequently(Fujii et al., 1997; Itoyama et al., 1994). These

studies however, may have been compromised by the use of antifibrinolytic medications in some patients.

The increase in fibrinogen may reflect the acute phase response. The increased availability of fibrinogen may be one of the explanations of the increased clot strength demonstrated using TEG (increased MA and G). Certainly the increase in these 3 variables follow a very similar time course. The increased fibrinogen concentration would increase plasma viscosity and increase the thrombotic potential. Certainly an increased fibrinogen concentration is a well-recognised epidemiological marker for an increased rate of myocardial infarction, ischaemic stroke and thrombosis. An increased fibrinogen gives an increased propensity for fibrin formation. During the acute phase reaction, there is a diversion of synthesis away from somatic and circulating proteins such as albumin, towards acute phase proteins such as C-reactive protein, haptoglobin, caeruloplasmin, α -1 antitrypsin and fibrinogen. It would be interesting to investigate if the increase in fibrinogen over time following SAH, was similar in rate to the increase of a non-coagulation based acute phase protein such as caeruloplasmin or α -1 antitrypsin. In this way I could investigate if other mechanisms specific to the coagulation pathways were responsible for part of the increased thrombotic potential and not just the acute phase response.

The routine coagulation tests PT, INR and APTT do not change over time following the SAH, and although there is a difference for the PT between the first and 3 month reading, its value is still comfortably within the standard reference range. It appears that these tests are insensitive to the changes observed in the TEG data, and in the plasma assays of coagulation and

fibrinolysis. It is interesting to note that it has been suggested that these tests are analogous to the R time in the TEG analysis. The R time was the only TEG variable in which no statistically significant change was observed following the haemorrhage. These results provide further confirmation that PT, INR and APTT are insensitive with regard to detecting hypercoagulability. A similar trend in PT and APTT was observed by Nina et al (Nina et al., 2001).

The results of the haematological indices from the full blood count are extremely interesting. It appears that the aim of treatment i.e. haemodilution and hypervolaemia is comprehensively achieved with the fluid regimen employed. The haematocrit falls from a mean value of 0.39 l/l in the first week, to 0.32 l/l in the third week (0.42 l/l at 3 months). The changes in haemoglobin concentration and RCC fall in line with this haemodilution. The platelet count initially falls, presumably as a result of haemodilution or the consumption of platelets in the arrest of haemorrhage, but then there is a secondary thrombocytosis and its level rises acutely for at least 21 days. This biphasic response in the platelet count following SAH has been reported previously (Fujii et al., 1997).

It is interesting to note that despite powerful evidence of haemodilution during the first 21 days following the SAH, both the fibrinogen concentration and the platelet count rise abruptly. Also, despite the effects of haemodilution on the circulating clotting factors and their cofactors, I have observed powerful evidence of hypercoagulability in the blood samples taken within 21 days of the ictus. The elevated changes observed in the plasma markers

TAT, P1+2, D-dimers and PAP, would have been even higher if these assays had been adjusted for haemodilution.

The acute rise in the WCC is another reflection of the acute phase response following SAH. Its initial rise and very slow decline over the first month has been observed previously(Fujii et al., 1997).

I have investigated the effect of operative intervention on the trend of increased coagulability following SAH. It might be expected that the stress of surgery, pain, anaesthesia and the release of thromboplastins from the brain substance would produce a further increase in coagulation profiles(Barrer, Ellison, 1977; Gibbs et al., 1992). Any further increase in coagulation would predispose to thrombosis, and potential ischaemic outcome. In the operative study, I observed little change in TEG parameters around the time of surgery. These findings are consistent with those of other authors investigating the changes at similar time periods around open abdominal and pelvic surgery in the absence of large volume blood loss(Benzon et al., 1994; Ng, Lo, 1996a). The only significant difference found was a small increase in MA and G, perhaps indicating some enhancement of platelet function or fibrinogen, during this time. Goh et al(Goh et al., 1997) observed hypercoagulation in intra and postoperative TEG samples in neurosurgical patients. The explanation for this difference is probably the extent of brain resection and amount of thromboplastin released. The group studied by Goh et al were undergoing brain tumour resections. Tuman et al(Tuman et al., 1987) observed a statistically significant hypocoagulable change in the TEG variables R, K, Angle and MA on induction of anaesthesia, which I did not observe.

I observed no statistically significant difference in the postoperative trend in TEG variables between the surgically and non-surgically treated patients. This suggests that surgery was not additive to the profile of increased coagulation observed in SAH patients. However, the trend was for the surgical patients to be more hypercoagulable. This may be explained by the fact that the surgically treated group had a more hypercoagulable initial mean when their first TEG sample was performed.

Thus, I have failed to identify a difference between the surgical and non-surgically treated groups. However, an alternative explanation for this may be in the mixture of the non-operative patients. This group comprises both the best (angiogram negative) and worst (those treated conservatively because of their poor condition and prognosis) patients in a SAH population. It may be that if a pure perimesencephalic haemorrhage population, or a grade-matched embolisation population, were compared with the surgical group, then a significant difference might have been found. Due to a limitation in numbers, these comparisons have not been possible in this study. It has been suggested that the outcome following embolisation is better than after intracranial surgery for cerebral aneurysms, and that the risks of ischaemia are less. It is only speculative at this time how the influence of these treatment modalities on coagulation might influence patient outcome.

It has been suggested by some authors, that there is an association between makers of haemostatic activation following SAH and clinical outcome (Fujii et al., 1995; Fujii et al., 1997; Itoyama et al., 1994; Nina et al., 2001; Shibahashi, Yamaura, 1991). These authors have attempted to correlate

markers of haemostatic activation such as TAT, with the incidence of DIND(Fujii et al., 1997; Itoyama et al., 1994; Nina et al., 2001; Shibahashi, Yamaura, 1991). Fisher et al(Fisher et al., 1980) correlated the incidence of DIND and subsequent patient outcome following SAH, to the initial blood load on the CT scan using a four point scale. This finding had been repeated in other studies(Allen et al., 1983; Kasuya et al., 1998). They hypothesised that, as the amount of blood increases on CT imaging, so the incidence of vasospasm and DIND also increases. In my study, I have tried to bring these theories together and correlate the blood load (measured by Fisher grade), with haemostatic activation (measured using TEG).

I failed to show any statistically significant relationship in the present study. However, the means for each TEG variable did become increasingly hypercoagulable from Fisher grades 1 through to 3. Fisher grade 4 patients did not follow this trend, and had a mean value roughly between those in grade 1 and grade 2 for each TEG variable.

Thus, it must be asked, have I failed to show an association because non exists, or have I failed to show it because of a problem with the study design? Potential problems with the study design are firstly that I have grouped the patients, and that each grade was not equally represented. I only recruited 3 grade 1 patients. In addition I have grouped together the grade 3 and 4 patients. There is good evidence that grade 4 patients do not always follow the outcome and ischaemia trends associated with Fisher grading. It has been suggested that the presence of intracerebral and intraventricular blood is not as bad as widely distributed subarachnoid blood (grades 2 and 3), when it comes to predicting ischaemic outcome(Grosset et

al., 1994; Itoyama et al., 1994; Jarus-Dziedzic et al., 2000). When grade 2 and 3 patients were separated and compared, the mean values for the grade 2 patients were hypocoagulable for every TEG variable studied, compared to the grade 3 patients. However, this failed to reach statistical significance. This suggests that a difference may be present, but that I have failed to recruit enough patients to find statistical significance.

The study of SAH patients has also found further evidence to support the findings of Gorton et al (Gorton et al., 2000), that TEG can identify sex differences in coagulation. This is also in agreement with the data acquired in my control studies (control study 2). The results of the study show that females and males differed in their TEG values when sampled within 14 days of a SAH. As with the control data no difference was observed for R time. This suggests that the first fibrin strands are generated at the same rate in males and females. However, for all the other variables studied, females were more procoagulant than males. Thus, following a SAH, females had faster rates of fibrin build-up and cross-linking and enhanced overall final clot strength. These results mirrored the control data. These findings lend more support to the idea that males and females differ in their coagulation activation and formation. These changes are presumably under some form of hormonal influence. Further discussion of these findings can be found earlier in control study 2. It has not been previously suggested that females may be at greater risk of thrombosis and cerebral ischaemia following SAH due to increased coagulation, but these findings warrant its further investigation.

I have not shown any significant variation in the coagulation response following SAH using TEG that is dependent on the age of the patient. It is

likely that the small change found in MA has occurred by chance as a result of multiple significance tests. This is in agreement with our control data (control study 2).

As a consequence of the observational nature of my data, it can not be excluded that, part of the associations observed could be explained by bias and confounding. In these studies, patients were not screened at entry for prothrombotic states.

It is interesting to speculate that the formation of microthrombi within the small cerebral vessels, associated with vasospasm and hypercoagulability, could predispose to DIND and ischaemic symptoms in SAH patients. Potentially antifibrinolytic, antiplatelet or anticoagulant medications may be indicated to improve the cerebral microcirculation at different times following the ictus and improve patient outcome. The role for TEG in directing this management is still to be established.

The aetiology of this hypercoagulable change must also remain highly speculative. It is possible that the changes simply reflect the acute phase response to a systemic injury. Alternatively, it may be a response to an intrinsic factor released by brain following haemorrhage such as thromboplastin, or blood breakdown products in the CSF. Another possibility is that it is due to factors specific to the cerebral endothelium. There is a focal area of damage to the endothelium at the site of aneurysm rupture, but perhaps the histological changes observed in the endothelium of vasospastic vessels also play a significant part in the activation of the coagulation response. It must also be considered whether the treatment protocols employed in the management of patients following SAH, such as

haemodilution or bed rest, may also be contributing to the observed coagulation changes.

Conclusions

One of the difficulties in studying hypercoagulability is the requirement to perform specific factor assays to document its presence. This is time consuming and expensive, with the results taking several hours or days. Thus, TEG appears to be a useful alternative to identify and study postoperative hypercoagulability. The technique is simple and inexpensive, and the results can be obtained within one hour. Moreover, TEG provides additional information about platelet function and the coagulation process that cannot be obtained from individual factor assays or routine laboratory coagulation tests. Individual factor assays are still required however, to detect specific clotting defects and to determine the mechanisms of coagulation changes.

Haemodilution is achieved in SAH patients using a fluid protocol of 3 litres of normal saline per day. Haemoglobin, red cell count and haematocrit fall to a similar degree for the first 21 days following a SAH.

Despite haemodilution, platelet count and fibrinogen concentration rise acutely for the first 21 days following a SAH. The platelet count response is biphasic.

SAH patients are hypercoagulable in the period following a SAH when compared with blood samples taken 3 months later in the same patient.

SAH patients become increasingly hypercoagulable for the first 21 days following the ictus.

Plasma assays of coagulation and fibrinolysis show that both of these processes are stimulated following a SAH. However, coagulation is proportionally stimulated to a greater extent to tip the balance towards this process for the first 21 days following a SAH.

The routine coagulation assays PT, APTT and INR do not change for the first 21 days following a SAH.

Operative intervention in the form of craniotomy and clipping of an intracerebral aneurysm appears to produce little change in the baseline coagulation process following a SAH.

I have failed to show an association between haemostatic activation measured using TEG and Fisher grade following SAH.

There are differences in the haemostatic response following SAH in males and females. Female patients show hypercoagulation in TEG variables in the first 14 days following a SAH compared to male patients.

Age appears to have little or no effect on haemostatic mechanisms measured using TEG following SAH.

Experimental study 6

The in vitro effects of haemodilution with crystalloid and colloid solutions on coagulation using thromboelastography

Introduction

Over the last 20-30 years there has been a remarkable improvement in the results reported after surgery for ruptured intracranial aneurysms. Operative mortality has fallen from over 20% to less than 5%(Hop et al., 1997; Maurice, Kitchen, 1994). However, despite considerable advances in diagnostic, surgical and anaesthetic techniques, and perioperative management, the outcome for patients with SAH remains poor, with overall mortality rates of at least 25% and significant morbidity among approximately 50% of survivors. When more detailed outcome assessments of SAH patients have been performed in structured studies, it is clear that very few patients return to their level of premorbid function following the ictus(Hop et al., 1998).

Despite recent advances in diagnosis and therapy, cerebral ischaemia remains the greatest treatable cause of morbidity and mortality in patients who survive the ictus and become within the limits of neurosurgical care(Mayberg, 1998).

Cerebral vasospasm remains a devastating medical complication affecting 30-70% of survivors of aneurysmal SAH(Al Yamany, Wallace, 1999). Cerebral vasospasm is the delayed narrowing of large capacitance arteries at the base of the brain after SAH, often associated with radiographic or cerebral blood flow evidence of diminished perfusion in the distal territory of the affected artery.

In about one half of cases, vasospasm is manifested by the occurrence of a delayed ischaemic neurological deficit (DIND), which may resolve or progress to cerebral infarction. In contemporary series, 15% to 20% of such patients suffer stroke or die from vasospasm despite maximal therapy(Haley et al., 1992; Longstreth et al., 1993). If vasospasm decreases cerebral blood flow below 35ml/100g/min, brain tissue pH will decrease, and severe acidosis is associated with neuronal injury.

A reduction in plasma volume following SAH may contribute to the development of cerebral ischaemia(Hasan et al., 1990). Therefore, fluid balance management is an essential task(van Gijn, Rinkel, 2001). Avoidance of hypotension, hypoxia and hypovolaemia becomes a primary aim.

One strategy employed to prevent DIND is prophylactic volume expansion. There is some evidence that this is associated with a reduction in ischaemic episodes in the short term following SAH(Rosenwasser et al., 1983), and that cerebral perfusion can be improved by a reduction in blood viscosity associated with haemodilution(Wood et al., 1984b). This may preserve cerebral blood flow and oxygen delivery to tissues with borderline viability(Wood et al., 1984a).

Although moderate haemodilution is thought to be beneficial, marked decreases in haematocrit (below 30%) should be avoided. This is because a reduction in the oxygen carrying capacity of the blood outweighs the benefit of increased perfusion, resulting in a decrease in cerebral oxygen delivery(Reasoner et al., 1996).

The main determinants of blood viscosity in clinical practice are the haematocrit and shear rate. Volume loading may be accomplished by

infusion of isotonic crystalloids, colloids, or red blood cells, aiming to maintain the haematocrit around 30%. It has become routine practice to manage SAH patients with 3 litres of normal saline every 24 hours, unless there are haemodynamic contraindications.

The evolution of treatment protocols for patients with SAH has been influenced by large, multicentre prospective, randomised, cohort analyses and, more recently, by multicentre prospective, randomised trials. Nevertheless, several accepted treatment modalities have not been substantiated by rigorous clinical scientific assessment.

Several reports from uncontrolled studies described the resolution of deficits from vasospasm following the elevation of blood pressure, volume expansion, and haemodilution, with improved outcome of vasospasm compared with historical controls(Awad et al., 1987; Kassell et al., 1982; Levy, Giannotta, 1991; Muizelaar, Becker, 1986; Origitano et al., 1990). However, the efficacy of hypertension/hypervolaemia/haemodilution (HHH) has not been demonstrated in controlled trials, and studies of cerebral blood flow after initiation of therapy have been equivocal(Fandino et al., 1999). In addition studies have not been performed to determine which component of this therapy (haemodilution versus hypervolaemia versus hypertension) is most important. Only a proportion of patients with vasospasm respond to H/H/H therapy, with stroke and death rates from vasospasm approaching 15% in the series with the best outcome.

Initiation of H/H/H therapy is associated with significant risk, including cardiac failure, electrolyte abnormalities, cerebral oedema, bleeding abnormalities, and rupture of an unsecured aneurysm(Amin-Hanjani et al., 1999). An

uncontrolled series suggested that therapy might be more effective if initiated prophylactically before the onset of symptoms (preferably after clipping of the aneurysm). Treatment is usually continued beyond the period of risk for vasospasm or until abatement of vasospasm by clinical or transcranial Doppler parameters.

The effects of volume expansion therapy have not been studied properly in patients with aneurysmal SAH. At present, there is no sound evidence for or against the use of volume expansion therapy in patients with aneurysmal SAH(Feigin et al., 2000).

In acute ischaemic stroke the notion of 'time is brain' has evolved in the acute management. Irreversible ischaemic brain damage evolves over hours and reperfusion can rescue tissue that is functionally inactive, but still viable (the ischaemic penumbra)(Muir, 2001).

Despite all the available measures to prevent and/or treat aneurysmal SAH induced vasospasm, it remains a clinical challenge for neurosurgeons, intensivists, and neuroradiologists(AI Yamany, Wallace, 1999; Fandino et al., 1999).

In this prospective, observational, in vitro study, we aim to ascertain if haemodilution 'per se' may have an intrinsic effect on coagulation, specific to the diluent itself, and thus may be contributing to the observed changes in coagulation following SAH.

Methods

Using opportunity sampling, blood was taken from 20 volunteer subjects: 10 male (mean age 31.4 yrs SD 5.1) and 10 female (mean age 30.6 yrs SD 10.9).

Citrated whole blood was sampled from each subject.

Method of sampling, exclusion criteria, consent and ethical approval has been described previously in control study 1.

A 3.6 ml volume of blood was taken into a 'blue-capped' Vacutainer polypropylene tube, containing 0.5 ml of 3.2% (0.105M) sodium citrate (pH 7.4) (Vacutainer™ [Ref 367914]). The sample was immediately mixed by gentle inversion of the tube 3 times and left to stand at room temperature for 30 minutes.

Following this time, 4 plain polypropylene tubes were taken into which were placed:

Tube 1. 0.952ml CWB

Tube 2. 0.952ml CWB + 0.408ml 0.9% NS

Tube 3. 0.952ml CWB + 0.408ml Gelfusion

Tube 4. 0.952ml CWB + 0.408ml HES

CWB = Citrated whole blood, NS = Normal saline, HES = 200000/0.5 Hydroxyethylstarch.

Thus, a 30% haemodilution had been achieved in each of tubes 2, 3 and 4. All tubes were gently inverted 3 times to mix the contents, and left to stand for a further 30 minutes at room temperature.

After this time TEG analysis was performed simultaneously on each of the 4 tubes, through 4 separate TEG channels in series. This enabled a direct comparison of the coagulation profiles of each of the 4 tubes in the 20 subjects studied. The method of TEG analysis has been previously described.

This study compares the TEG coagulation profiles in an undiluted (control) sample (Tube 1), with a 30% crystalloid haemodilution (Tube 2), and a 30% haemodilution of two different colloid solutions (Tubes 3 and 4).

The advantages of performing an in vitro study on healthy volunteers, are that other factors which might of affected coagulation in SAH patients are excluded, such as: stress, acute phase reaction, anaesthesia, surgery, blood loss and endothelial injury.

Statistical analysis

The mean, standard deviation and 95% confidence intervals were calculated for each TEG variable in each of the groups.

To assess the effect crystalloid and colloid haemodilution on coagulation, multiple two-sample paired t-tests were used for comparison of TEG variables between the different groups (Appendix VII).

Results

R time

The R time is the TEG variable least affected by haemodilution in this study. This is interesting as it is the TEG variable most closely associated with the value of the routine coagulation tests PT and APPT, and stops at the point where the first fibrin strands start to form in the clot. It can be seen from Appendix VII, Tables 45 to 49, and Figure 50 that the only significant differences found in R were associated with the Gelfusion sample which had a significantly shortened R time compared to all other samples. This data suggests that the routine tests PT and APPT would not have picked up the differences in coagulation profiles identified with TEG in the following sections.

K time

The K time was significantly shortened by haemodilution with normal saline and Gelfusion, and significantly lengthened by haemodilution with HES (Appendix VII, Tables 45 to 49, and Figure 51).

Angle

The Angle was significantly increased with Gelfusion haemodilution, and significantly decreased with HES haemodilution. These differences were also observed in comparison to the normal saline sample (Appendix VII, Tables 45 to 49, and Figure 52). Although the saline dilution increased the mean Angle compared to the undiluted sample, this increase was not statistically significant.

MA

All fluids used to provide haemodilution significantly reduced MA. There was a significantly greater reduction with HES than normal saline, and a significantly greater reduction with normal saline than Gelfusion (Appendix VII, Tables 45 to 49, and Figure 53).

Shear elastic modulus G

All fluids used to provide haemodilution significantly reduced G. There was a significantly greater reduction with HES than either Gelfusion or normal saline haemodilution (Appendix VII, Tables 45 to 49, and Figure 54).

	R	K	ANG	MA	G
Mean	658.9	215.8	47.175	57.025	6643.8
Standard Error	27.406	14.964	1.76899	1.12301	353.22
Median	682	215.5	47	56.5	6494.5
Mode	#N/A	145	42.5	56.5	6494.5
Standard Deviation	122.56	66.919	7.91114	5.02225	1579.6
Sample Variance	15022	4478.2	62.5862	25.223	2E+06
Kurtosis	-0.267	0.3442	-1.13143	0.55856	1.5313
Skewness	-0.436	0.6228	-0.08463	0.48273	1.1892
Range	432	269	27.5	20	6101
Minimum	428	115	33.5	48	4524
Maximum	860	384	61	68	10625
Sum	13178	4316	943.5	1140.5	132875
Count	20	20	20	20	20
Confidence Level(95.0%)	57.361	31.319	3.70253	2.35049	739.29

Table 45. Summary of TEG statistics for the undiluted samples (Tube 1).

	<i>R NS</i>	<i>K NS</i>	<i>ANG NS</i>	<i>MA NS</i>	<i>G NS</i>
Mean	667.95	193.75	48.625	51.725	5658.7
Standard Error	30.113	9.1921	1.38168	0.7767	220.16
Median	690.5	183	49.5	51	5526.5
Mode	499	180	41.5	49.5	5526.5
Standard Deviation	134.67	41.108	6.17907	3.47349	984.59
Sample Variance	18136	1689.9	38.1809	12.0651	969424
Kurtosis	-0.622	0.4779	-0.17508	-0.06148	-0.5
Skewness	0.2733	0.7351	-0.21787	0.63724	0.6886
Range	481	165	24.5	13	3312
Minimum	467	125	35.5	46.5	4346
Maximum	948	290	60	59.5	7658
Sum	13359	3875	972.5	1034.5	113175
Count	20	20	20	20	20
Confidence Level(95.0%)	63.028	19.239	2.8919	1.62564	460.8

Table 46. Summary of TEG statistics for the samples with a 30% haemodilution with 0.9% normal saline (NS)(Tube 2).

	<i>R Gel</i>	<i>K GEL</i>	<i>ANG GEL</i>	<i>MA GEL</i>	<i>G GEL</i>
Mean	607.85	180.7	54.15	54.15	5904.2
Standard Error	18.967	11.826	1.49345	1.02026	255.89
Median	621.5	175	54.5	53.25	5526.5
Mode	667	180	55.5	51	5204
Standard Deviation	84.822	52.887	6.67891	4.56272	1144.4
Sample Variance	7194.8	2797.1	44.6079	20.8184	1E+06
Kurtosis	-0.369	1.7473	1.29271	-0.56333	0.8742
Skewness	-0.485	1.2258	-0.46691	0.4066	1.0565
Range	310	215	29	16.5	4365
Minimum	441	100	38	47.5	4524
Maximum	751	315	67	64	8889
Sum	12157	3614	1083	1083	118084
Count	20	20	20	20	20
Confidence Level(95.0%)	39.698	24.752	3.12583	2.13542	535.58

Table 47. Summary of the TEG statistics for the samples diluted 30% by Gelfusion (GEL)(Tube 3).

	<i>R HES</i>	<i>K HES</i>	<i>ANG HES</i>	<i>MA HES</i>	<i>G HES</i>
Mean	679.75	238.4	44.825	47.8	4649.2
Standard Error	27.8	11.59	1.35203	0.87389	164.4
Median	668	237.5	44.75	48	4662
Mode	629	215	43	50.5	5101
Standard Deviation	124.33	51.833	6.04648	3.90816	735.2
Sample Variance	15457	2686.7	36.5599	15.2737	540518
Kurtosis	0.6431	0.8303	0.14535	1.80786	2.2666
Skewness	0.2835	0.7376	0.09766	-0.12562	0.5402
Range	516	205	22.5	18.5	3498
Minimum	423	150	33.5	38.5	3130
Maximum	939	355	56	57	6628
Sum	13595	4768	896.5	956	92983
Count	20	20	20	20	20
Confidence Level(95.0%)	58.186	24.259	2.82984	1.82907	344.08

Table 48. Summary statistics of TEG variables for the samples 30% diluted with hydroxyethylstarch (HES)(Tube 4).

t test	R1R2	R1R3	R1R4	R23	R24	R34
P value	0.39	0.02	0.24	0.02	0.32	0.003
	K1K2	K1K3	K3K4	K2K3	K2K4	K3K4
P value	0.048	0.01	0.05	0.14	0.0001	0.0003
	A1A2	A1A3	A1A4	A2A3	A2A4	A3A4
P value	0.13	0.00007	0.0495	0.0003	0.0008	3E-06
	MA1MA2	MA1MA3	MA1MA4	MA2MA3	MA2MA4	MA3MA4
P value	6E-06	0.004	1E-10	0.02	0.00008	1E-08
	G1G2	G1G3	G1G4	G2G3	G2G4	G3G4
P value	0.004	0.004	3E-07	0.22	0.00001	1E-06

Table 49. Summary of t-tests between TEG variables R, K, Angle (A), MA and G for undiluted and diluted samples.

- 1 = Undiluted citrated whole blood
- 2 = CWB with a 30% haemodilution with 0.9% normal saline
- 3 = CWB with a 30% haemodilution with Gelfusion
- 4 = CWB with a 30% haemodilution with HES

Figure 50. Scatter plots of R values with means and 95% confidence intervals for undiluted and diluted samples.

Wb = Citrated whole blood, ns = Normal saline, gf = Gelfusion, hs = Hydroxyethyl starch.

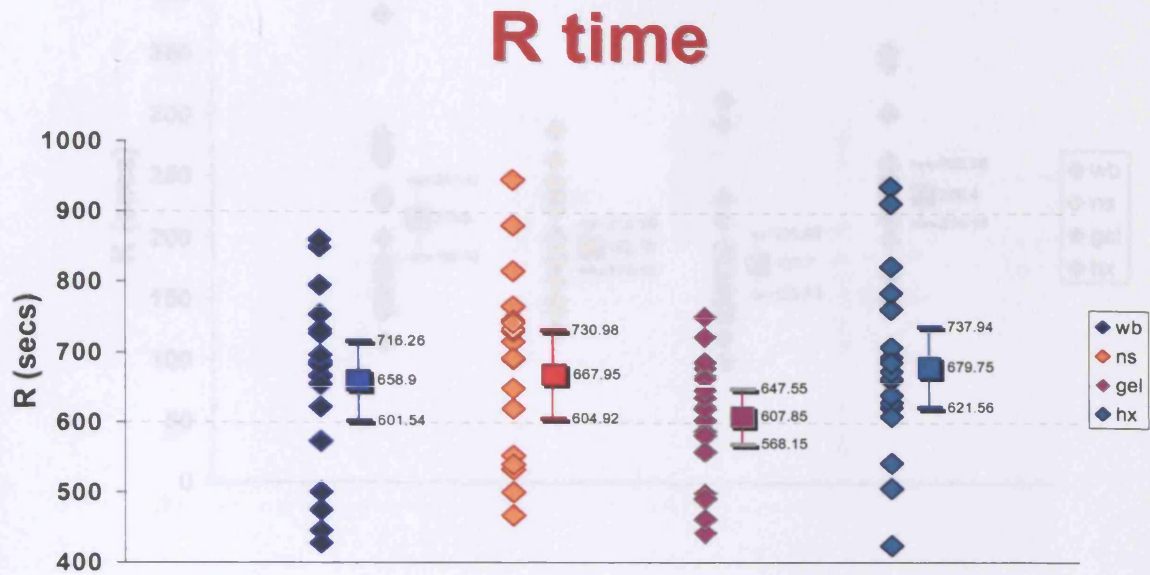


Figure 51. Scatter plots of K values with means and 95% confidence intervals for undiluted and diluted samples.

Figure 50. Scatter plots of R values with means and 95% confidence intervals for undiluted and diluted samples.

Wb = Citrated whole blood, ns = Normal saline, gel = Gelfusion, hx = Hydroxyethyl starch.

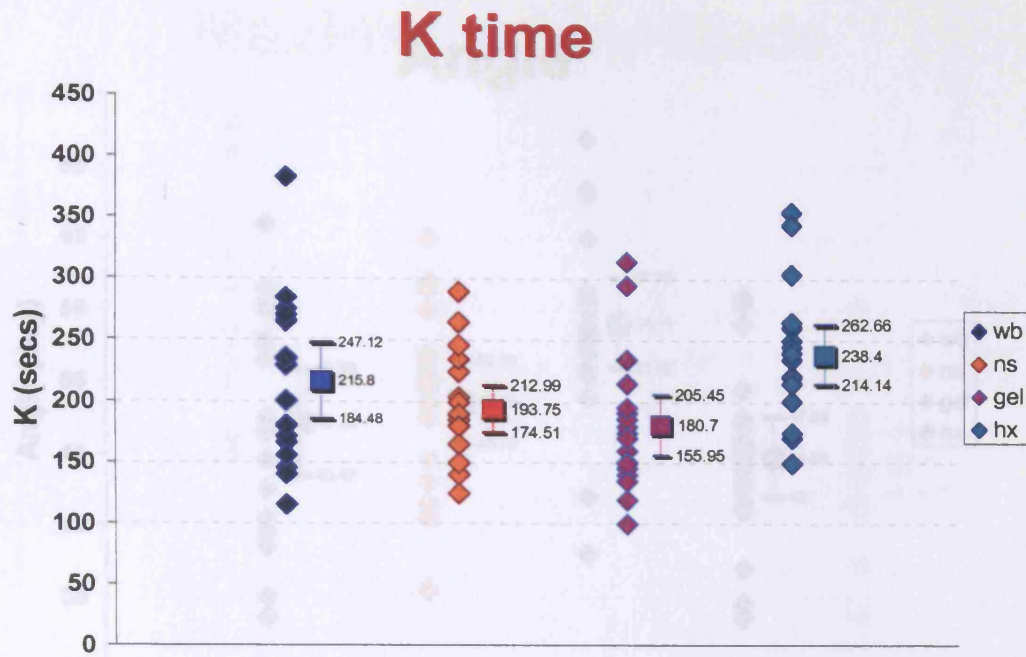


Figure 51. Scatter plots of K values with means and 95% confidence intervals for undiluted and diluted samples.

Wb = Citrated whole blood, ns = Normal saline, gel = Gelfusion, hx = Hydroxyethyl starch.

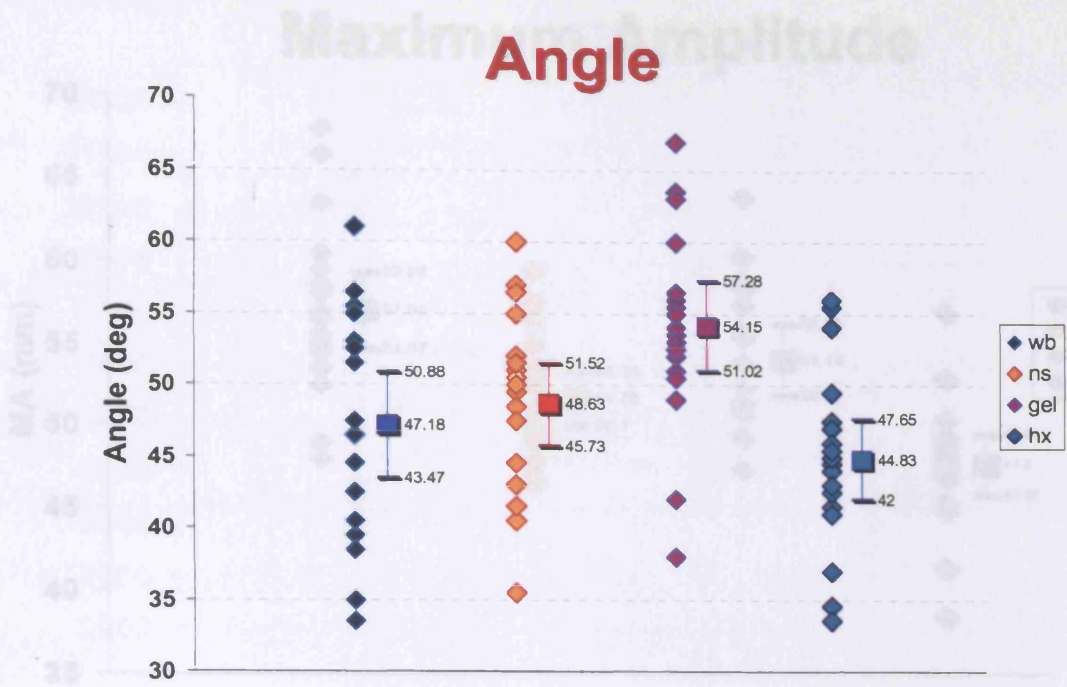


Figure 52. Scatter plots of Angle values with means and 95% confidence intervals for undiluted and diluted samples.

Wb = Citrated whole blood, ns = Normal saline, gel = Gelfusion, hx = Hydroxyethyl starch.

Maximum Amplitude

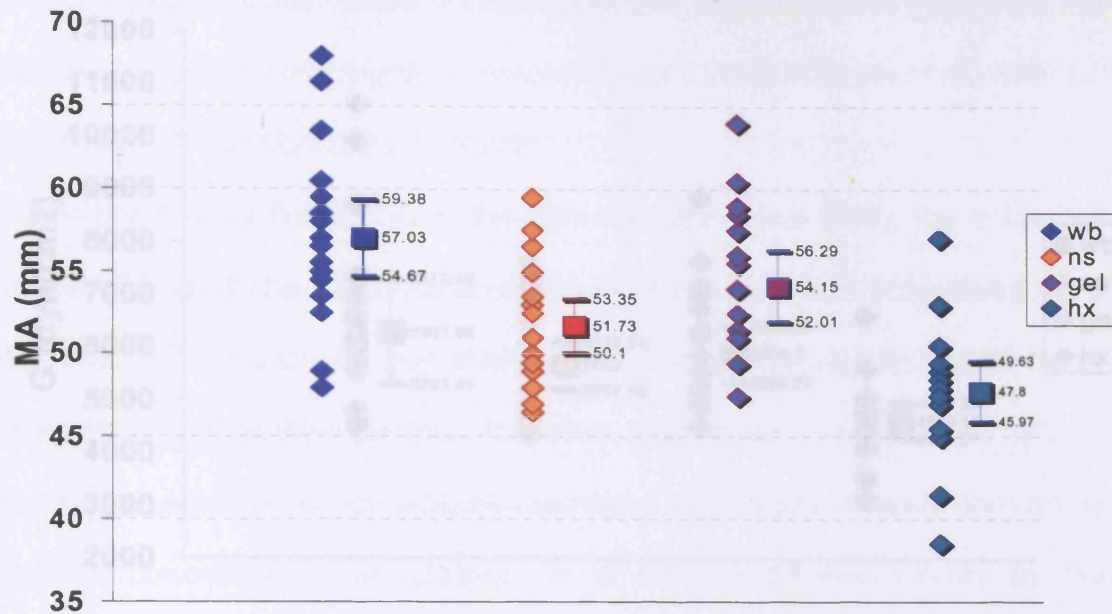


Figure 53. Scatter plots of MA values with means and 95% confidence intervals for undiluted and diluted samples.

Wb = Citrated whole blood, ns = Normal saline, gel = Gelfusion, hx = Hydroxyethyl starch.

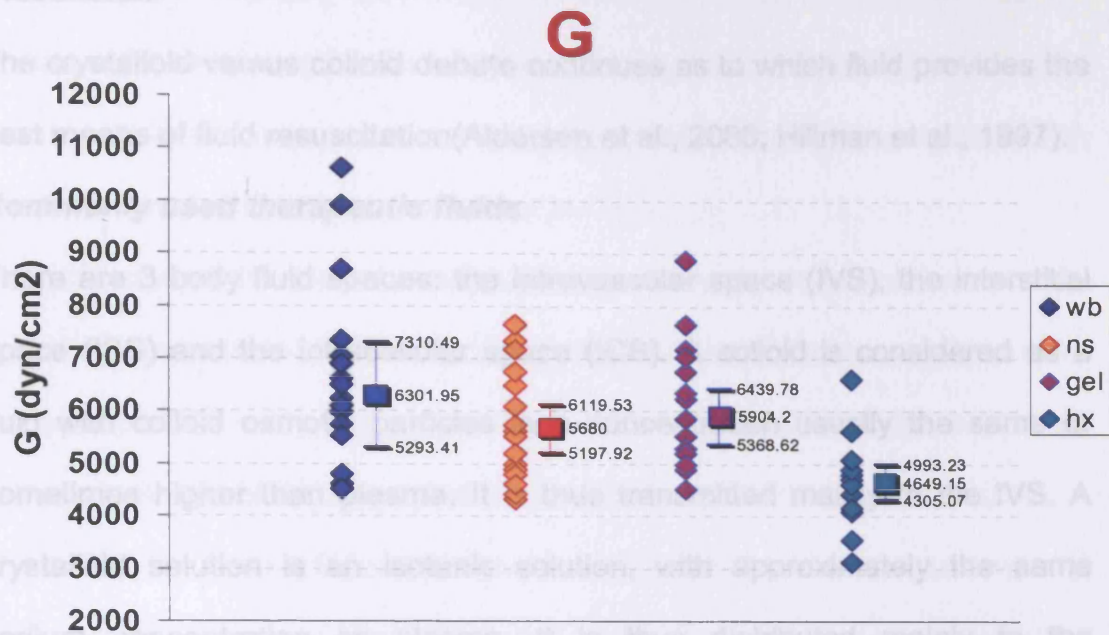


Figure 54. Scatter plots of G values with means and 95% confidence intervals for undiluted and diluted samples.

Wb = Citrated whole blood, ns = Normal saline, gel = Gelfusion, hx = Hydroxyethyl starch.

Discussion

The crystalloid versus colloid debate continues as to which fluid provides the best means of fluid resuscitation(Alderson et al., 2000; Hillman et al., 1997).

Commonly used therapeutic fluids

There are 3 body fluid spaces: the intravascular space (IVS), the interstitial space (ISS) and the intracellular space (ICS). A colloid is considered as a fluid with colloid osmotic particles in a concentration usually the same or sometimes higher than plasma. It is thus transmitted mainly to the IVS. A crystalloid solution is an isotonic solution, with approximately the same sodium concentration as plasma. It is thus distributed mainly to the extracellular space (IVS + ISS). Crystalloid solutions have become the first line fluid for resuscitation in most forms of hypovolaemia.

The ideal crystalloid for use in neurosurgery is controversial. Lactated Ringer's solution and 0.9% normal saline, both have theoretical disadvantages, but there is no patient outcome data to advocate one over the other. Ringer's solution is mildly hypoosmolar when compared to plasma, whereas 0.9% normal saline has an osmolarity of 308 mOsm/l, slightly greater than plasma. If large amounts of intravenous fluids are required, there may be a significant decrease in osmolarity with the use of Ringer's, which might contribute to undesirable cerebral oedema. Thus, some authors have suggested that its use be limited in neurosurgical procedures. However, experimental animal data has shown a survival advantage after resuscitation with Ringer's when compared with normal saline following haemorrhagic shock. Also, large volumes of 0.9% normal saline has been associated with the precipitation of a hyperchloraemic acidosis(Williams, 1998).

Colloid solutions usually have the same colloid osmotic pressure as plasma. They are therefore confined mainly to the IVS. They are therefore the most efficient fluid for resuscitation during hypovolaemia, and compared with crystalloids, result in little expansion of the ISS.

Because crystalloids have isotonic concentrations of sodium and no oncotic particles, they are distributed equally throughout the whole extracellular fluid. Because the ISS comprises at least two-thirds of the extracellular fluid, most crystalloid is distributed to the ISS rather than to the circulating volume.

The ICS is the largest fluid space, containing more than half the body's total water. It is separated from the ISS by cell membranes, which allow potassium to enter the cells while actively exporting sodium. Thus, salt containing solutions such as crystalloids are largely excluded from the ICS. Solutions such as 5% dextrose, which are distributed equally over all body fluid spaces, can be effectively used to rehydrate the cell, because the majority is distributed to that space(Hillman et al., 1997).

Colloids are solutions that contain natural (albumin) or synthetic molecules (HES and dextran) that are relatively impermeable to the vascular membrane. Albumin (molecular weight 66300 to 69000) is the major plasma protein active in osmosis and is responsible for 80% of the plasma colloid pressure. Albumin has a half-life of 16 hours. Albumin may lower serum ionised calcium producing a negative inotropic effect on the myocardium and can cause minor changes in PT and APPT.

HES is a synthetic colloid derived from corn starch. It consists of a heterogeneous group of molecules ranging in molecular weight from under 10000 to over 1000000. Because of the heterogeneity of the HES molecule,

its pharmacokinetic properties are complex. Its disappearance from the body is governed by the extent of hydroxyethylation, and the molecular weight of the starch. Smaller molecules (<50000) will be readily excreted in the urine, whereas the larger molecules will undergo enzymatic degradation before elimination. The advantages of HES over other synthetic colloid solutions include a lower incidence of side effects and allergic reactions, stability upon prolonged storage, immediate availability, no dependence on human blood donation, no risk of sexually transmitted diseases and lower cost.

Dextran solutions are glucose polymers fractionated to provide a low molecular weight and a higher molecular weight product. Dextran 70 has an average molecular weight of 70000. The half-life of these solutions increase as the molecular weight rises. Dextrans with a molecular weight exceeding 55000, have a half life exceeding 12 hours.

In recent years there has been increasing awareness of the potential side effects of allogeneic blood products. With increasing use of artificial plasma expanders, their influence on blood coagulation may become clinically relevant. However, assessing the influence of various plasma expanders on blood coagulation is difficult because the observed effect may be a result of an intrinsic effect of the plasma expander molecules in addition to an effect of haemodilution on coagulation per se(Egli et al., 1997).

Haemodilution with intravenous fluids will decrease the concentration of clotting factors and platelets in the blood, and intuitively it would be anticipated this should induce some impairment of coagulation. However, these changes do not result in increased bleeding unless the dilution is profound(Mortier et al., 1997). It appears that the reserve capacity of the

coagulation system is so great, that dilution is of no consequence unless large volumes are used.

In this study we have compared the effects on coagulation of a 30% haemodilution in vitro with a crystalloid 0.9% normal saline, and two colloid solutions Gelfusion and HES 200/0.5.

Experimental and clinical evidence of haemodilution on coagulation

Ruttmann and colleagues(Ruttmann et al., 1996) compared whole blood TEG with samples diluted by 20% with 0.9% saline, and a modified gelatin colloid solution (Haemaccel). The R and K times were reduced relative to control in both diluted groups. The angle was increased in both groups compared to control, while MA was unchanged in the Haemaccel diluted group. The authors concluded that haemodilution per se increases the coagulability of whole blood in vitro, but that saline haemodilution had a more profound effect on final clot strength. The mechanism by which haemodilution may promote coagulation is far from clear.

When perioperative blood loss is replaced partly with plasma expanders, impairment in haemostasis may be anticipated(Karoutsos et al., 1999). Karoutsos and colleagues(Karoutsos et al., 1999) investigated the effect of different plasma expanders on coagulation measured immediately following total hip replacement. They found that the postoperative TEG of patients who received gelatin as a blood substitute showed significant hypercoagulability compared with control tracings and with the albumin and HES groups. In this study gelatin affected the onset of the clotting process (R, K, and Angle) but not the clot strength (MA). A hypothesis for this normal MA could involve interaction of fibronectin and gelatin, with clot-incorporated gelatin-fibronectin

complexes impeding polymerisation of fibrin. Although TEG demonstrated a state of hypercoagulability, no significant change in standard tests of haemostasis was observed. This in vivo study however, was compromised as each studied patient was also given the crystalloid Ringer's lactate, and some patients received subcutaneous heparin.

In vivo work has demonstrated that not all intravenous fluid regimens effect TEG coagulation profiles in the same way. Martin et al showed that the colloids Hextend and Hexpan were hypocoagulable compared to the crystalloid Ringers lactate(Gan et al., 1998; Martin et al., 1999).

Kapiotis et al(Kapiotis et al., 1994) used 10 healthy male volunteers to investigate the in vivo infusion of 500ml HES 200/0.5 or 500ml human albumin 5% on coagulation. After a washout period of 4 weeks, subjects crossed over to the alternate treatment. Blood samples were taken immediately before infusion and 20, 45, 75, 105, 165, 285, 405, and 1485 minutes after the infusion started. Haematocrit, fibrinogen, APPT, factor VIII:C, TAT complex, D-Dimer, tPA, uPA, PAI, and PAP complex, were all measured. Except for factor VIII:C levels, which were significantly lower in the HES 200/0.5 group (the albumin group showed no change), no other significant differences in the plasma levels were detected between the 2 groups.

During this study, infusion of albumin and HES caused a significant decrease in haematocrit. This had returned to normal by 1485 minutes. APPT was significantly prolonged until 105 minutes. TAT levels significantly increased until 105 minutes. Fibrinogen levels fell for the first 45 minutes, but had almost returned to normal by 1485 minutes. D-Dimer levels decreased until

45 minutes. The tPA and uPA levels were decreased until 105 minutes. No change in PAP levels was observed. Plasma levels of PAI were reduced significantly in both groups at 165, 285, and 405 minutes.

However, in vitro work by Ruttman and colleagues (Ruttman, James, 1999) has shown that haemodilution with 0.9% saline increased coagulability of whole blood, as measured by TEG variables (not MA). The presence of an antiplatelet agent (aspirin) had no effect on this observation, and they concluded that the hypercoagulation was not mediated via a cyclo-oxygenase pathway (Ruttman, James, 1999). These studies showed a decrease in antithrombin III with both crystalloid and colloid haemodilution, and increased platelet aggregation in the crystalloid group, suggesting that both crystalloid and colloid enhanced the consumption of fibrinogen and that saline increased the aggregation of platelets. This effect was maintained even after the salt solutions were buffered to normal pH at 37°C or the addition of a protein poor body fluid such as cerebrospinal fluid (CSF) (Ruttman et al., 1999). They concluded that the electrolyte and acid-base composition of the diluting fluid had no effect on the observed hypercoagulability that is produced with crystalloid haemodilution. They suggested that the trigger must be some aspect related to haemodilution and not to any biochemical abnormalities in the diluting fluid.

The results with CSF were extremely interesting and showed a procoagulant effect in excess of that expected through haemodilution. They suggest that the procoagulant effect of CSF should arrest bleeding into this space more quickly than into other body cavities such as the epidural space.

In an earlier study, Ruttman et al (Ruttman et al., 1998) investigated the effects of haemodilution with either saline or HES (200/0.5), on blood coagulation in healthy volunteers in vivo. Standard coagulation tests (packed cell volume (PCV), platelets, PT, APPT, fibrinogen, antithrombin III, bleeding time and platelet aggregation) and TEG were performed before and after administration of either 1000mls of 0.9% saline or HES, intravenously over 30 minutes. Dilution of PCV and platelet concentrations as a result of volume load were 9% in the saline group and 19% in the HES group. Reductions in fibrinogen (18.6% and 28.8%) and antithrombin III (25.5% and 37.8%) were significantly greater than could be explained by haemodilution alone in both groups. Indices of platelet aggregation were significantly enhanced by saline dilution, but not by HES, which inhibited epinephrine induced aggregation and prolonged bleeding time. TEG in the saline group showed significantly shortened R and K times (24% and 26% respectively), and increased angle (24%) and MA (6%). In this study, HES haemodilution decreased MA (11%) but did not affect other TEG variables. The authors concluded that the overall procoagulant response of saline haemodilution could possibly be explained by enhancement of thrombin formation. The evidence for this being the depletion of circulating antithrombin III levels below what could be explained by haemodilution alone.

The mechanisms by which these effects may occur are unknown. However, it is interesting to observe that there may have been a survival benefit for the evolution of such a mechanism. One may speculate that when internal haemodilution occurs after blood loss, enhanced coagulability may prevent further haemorrhage in the wild state, and this could offer a survival benefit.

To avoid the risks associated with transfusion of homologous blood products and to limit the high cost of albumin solutions, artificial colloid solutions represent an alternative for intraoperative blood loss replacement. Mortier and colleagues (Mortier et al., 1997) tested the effect of profound haemodilution to 50% of various colloid solutions and compared them to controls using TEG. In these extreme conditions gelatin 4% had no significant effect, hydroxyethylstarch prolonged K time and reduced angle and MA, however dextran 40 10% had the most profound effect, often resulting in a straight line on the TEG. Interestingly there was a significant shortening of the R time in both the gelatin 4% and hydroxyethylstarch preparations reflecting increased intrinsic coagulation. Thus, although the tendency of colloids is towards the hypocoagulable, not all colloids act equally and some products produce contradictory effects on different TEG variables at 50% dilution. While haemodilution obviously reduces the concentrations of platelets and clotting factors, it is perhaps the effect of some colloids on platelet adhesiveness and aggregation that is clinically more significant, or a reduction in the cross linkage of fibrin (Mortier et al., 1997).

Egli et al (Egli et al., 1997) compared in vitro, the effect of haemodilution (30% and 60%) with saline 0.9%, HES 200/0.5, gelatin and 5% albumin in 96 patients using TEG. Haemodilution with HES, gelatin, albumin and saline resulted in a similar decrease in haemoglobin concentration. Compared with native blood, progressive haemodilution with HES, gelatin and albumin did not affect the R time, but compromised the rapidity of clot formation, as manifested by an increase in K and a decrease in angle. With HES, this was

significant at 30% haemodilution; with gelatin and albumin, significant changes were observed only at 60% haemodilution. The strength of the clot (MA) was decreased by HES, gelatin and albumin at 30% and 60% haemodilution. Thus, HES had the most negative effect on K, angle and MA. Clot lysis at 30 and 60 minutes was not affected by haemodilution.

Haemodilution with saline at 30% reduced R and K time and increased angle. At 60% haemodilution all 3 variables recovered. MA was compromised at 60% haemodilution, and clot lysis at 30 and 60 minutes showed a progressive decrease with increasing haemodilution.

Dextran 40 has been studied in vitro. It has been used clinically to prevent thromboembolism. It binds to platelets and preferentially to activated platelets. It stimulates platelets and increases fibrinogen binding. It has been hypothesised that dextran 40 may exert a beneficial antiplatelet/antithrombotic effect in vivo by inhibiting the recruitment of activated platelets to the growing thrombus.

HES is a modified natural polymer of amylopectin with volume expansion properties. Its physical and chemical characteristics are defined by the degree of hydroxyethylation, which is the major determinant of plasma half-life, and also by its molecular weight, which determines colloidal activity. Solutions of native starches are unstable and rapidly hydrolysed by plasma amylases. With minimal substitution of hydroxyethyl radicals on glucose units, solution stability is excellent, but hydrolysis is rapid and half-life very short. Kinetics of elimination is improved by higher substitution (Treib et al., 1999).

It has been suggested that the mean molecular weight of HES solutions may be an important characteristic in determining their effects on blood coagulation during haemodilution. Jamnicki et al (Jamnicki et al., 1998) compared the effects of the recently developed HES solution with a molecular weight of 130kD (HES 130) compared with the currently widely used HES 200 kD (HES 200) on in vitro blood coagulation using TEG. They compared control samples, with 0.9% saline dilution and blood diluted with HES 130 and HES 200, to achieve 30% and 60% haemodilution. All samples were celite activated. Both HES solutions significantly compromised blood coagulation, as seen by an increase in R and K time, and a reduction in angle and MA. There was no significant difference between HES 130 and HES 200 diluted blood. When analysing the intrinsic HES effect by taking haemodilution with 0.9% normal saline into account, progressive haemodilution with both HES solutions resulted in an increasing clot lysis. Again there was no difference in the HES 130 and HES 200 diluted samples. Changes with 60% haemodilution were greater than with 30% haemodilution. HES decreases platelet adhesiveness and aggregation, and decreases both factor VIIIc and factor VIII:RC levels (Egli et al., 1997; Mortier et al., 1997; Strauss, 1981), it is not surprising that dilution with this colloid causes marked changes in the TEG.

In the presence of HES, faster transformation of fibrinogen to fibrin has been shown (Carr, Jr., 1986; Strauss et al., 1985). The fibrin was shown to be less stable as evidenced by a shortened urokinase-activated clot lysis time suggesting enhanced fibrinolysis (Carr, Jr., 1986; Strauss et al., 1985). Also, gelatin has been shown to be incorporated into developing blood clots and

thus potentially interfere with fibrin polymerisation(Mardel et al., 1996). In this study both succinylated gelatin (Gelofusine) and 3.5% polygeline solution (Haemaccel) demonstrated a reduction in clot weight and shear elastic modulus strength as measured by TEG. After clot formation the remaining serum was negative for fibrinogen and cross-linked FDPs, suggesting these physical changes reflect an alteration in structure of the coagulum when formed in the presence of gelatin colloids. This was supported by scanning electron microscopy. The widespread mesh pattern of fibrin strands seen in normal blood clot was retained in 0.9% saline control, while this reticular network was much reduced in clots containing gelatin colloids. Gelatin has been shown to bind with fibronectin, and polygeline infusion has been shown to decrease plasma fibronectin concentrations in vivo. Mardel et al(Mardel et al., 1996) suggested that gelatin based products may become incorporated into developing clots and reduce the function of fibronectin in forming covalent cross-linkages and normal covalent associations with fibrin, thus interfering with polymerisation of fibrin monomers.

Jamnicky's study(Jamnicky et al., 1998) found no difference in the coagulation effects induced by the different molecular weight solutions. However, HES molecules are usually characterised by their degree of substitution (defined as the average number of hydroxyethyl groups per glucose moiety), and their C2 to C6 ratio, which describes the ratio of the hydroxyethyl groups situated at the C2 and C6 positions of the glucose molecule, in addition to their mean molecular weight. These structural aspects of the HES molecule also modulate the influence on blood coagulation. The greater the molecular

weight, the higher the degree of substitution and the C2 to C6 ratio, the more a particular solution may compromise coagulation(Konrad et al., 2000).

Despite different molecular weights of 130 kD and 200 kD, no coagulation difference was observed. An explanation may be that the degree of substitution was also minimally different (0.4vs 0.5), and more importantly, there was a significant difference in the C2 to C6 ratio. HES 130/0.4 has a higher C2 to C6 ratio (11.2) than HES 200/0.5 (5.1). Because a high C2 to C6 ratio is associated with an exaggerated blood coagulation compromising effect, it is conceivable that the effects of a lesser mean molecular weight and a higher C2 to C6 ratio of HES 130/0.4 resulted in a blood coagulation-compromising potency, similar to the higher molecular weight but in a lower C2 to C6 ratio of HES 200/0.5.

In this study 30% haemodilution with 0.9% saline induced a significant decrease in R and MA, and a significant increase in angle. There was no change in K compared with native blood. LY30 was unchanged, and LY60 decreased slightly. However, 60% haemodilution with 0.9% saline induced a significant increase in R and K, and a significant decrease in angle and MA. Both LY30 and LY60 decreased compared with native blood.

Using a different measure of coagulation, the Sonoclot, Konrad et al(Konrad et al., 2000) compared the effect of 3 different molecular weight HES preparations (70000, 130000, 200000kD). At a 33% haemodilution all preparations interfered with the early stage of coagulation, expressed as clot rate. All interfered with haematocrit, fibrinogen, red cell and platelet count equally. Interestingly, HES 130000, showed a faster clot formation rate and better clot retraction than the other preparations on the Sonoclot.

Jamnicky et al went on to compare HES 200 kD with low molecular weight HES 70 kD(Jamnicky et al., 2000). They found that low molecular weight HES compromised blood coagulation slightly less than HES 200 however, it was unclear whether this was clinically relevant. A slightly greater compromise with HES 200 was found for APPT, factor VIII, vWF, MA and angle on the TEG. No difference was observed for PT, fibrinogen, platelet count, and R, K, LY30 and LY60 on the TEG.

In 1980, Janvrin et al(Janvrin et al., 1980) using a technique called the Biobridge, suggested that blood clotted faster when diluted with saline. They studied 60 laparotomy patients, 30 received intravenous fluids before, during and following surgery and 30 did not. The patients given intravenous fluids became significantly more haemodilute and hypercoagulable. A deep vein thrombosis was observed in 30% of patients given intravenous fluids and in only 7% of those who were not. The patients with DVT had significantly shorter coagulation times at the end of surgery than the patients without DVT.

Using TEG, Heather et al(Heather et al., 1980) used this finding to propose the 'saline dilution test'. They suggested that the degree to which the coagulability of an individual patient's blood increased by such a dilution, was directly related to the patient's risk of developing a postoperative DVT.

As part of this study(Heather et al., 1980) a preliminary experiment was carried out to determine the dilution at which subsequent coagulability measurements should be performed. Freshly drawn whole blood specimens from a series of preoperative patients were progressively diluted in 3 stages with increasing volume of normal saline, and the TEG coagulability index of

the undiluted blood and each of the 3 diluted specimens was determined. Although there was a large variation between patients in the percentage increase in the coagulability index at comparable dilutions, all samples tested showed a maximum coagulability at whole blood concentrations of 75-85% of normal, which declined with further dilution.

The authors found that the mean percentage increase in coagulability index shown after dilution of the blood of those patients subsequently developing a DVT was 55 +/- 16%. The corresponding increase for patients who did not develop a DVT was 19 +/- 11%. In 8 of the 10 patients whose index increased by more than 30% after dilution developed a DVT, while none of the remaining 10 whose blood showed an increase in the index of less than 30% did so. The authors suggested that this test might predict the maximum potential coagulation to in vivo haemodilution.

HES is an appealing alternative to plasma protein fractions (PPF) in the treatment of cerebral vasospasm because it has an extended shelf life, is not subject to shortages that may affect the supply of blood products, may be given to patients who do not take blood products on religious grounds, and carries no risk of transmitting blood borne diseases. The risk of anaphylactic reactions is also very small. Trumble et al (Trumble et al., 1995) compared the use of HES and PPF in the treatment of acute vasospasm in 26 patients following aneurysmal SAH in patients who had undergone surgery to clip the identified aneurysm. Both colloids were administered at a median rate of 50 cc/hour. Post treatment PT was slightly above pre-treatment levels in both groups, whereas platelet and fibrinogen levels revealed no trend. The APPT

was found to be significantly elevated in all patients who received HES, but was not significantly altered in patients who received PPF.

HES serves as potent volume expander, with the initial intravascular component comparable to the infused volume. This agent decreases serum albumin and globulin in a dilutional manner. In the treatment of vasospasm the literature has suggested the use of various plasma substitutes for optimum haemodynamic and haemorheological effects in the cerebral circulation. Previous studies showing a reduction in infarct size and increased cerebral blood flow with these agents(Kroemer et al., 1987). The aetiology of this improvement is unclear, but suggested theories have included haemodilution, decreased plasma viscosity, increased cardiac output, or a mild elevation of clotting time. The mild elevation of clotting time by HES has been ascribed to the precipitation by HES of factor VIII moieties i.e., procoagulant factor VIII, von Willebrand factor, and factor VIII-related ristocetin cofactor(Baldassarre, Vincent, 1997; Korttila et al., 1984; Trumble et al., 1995). This effect may not be concentration dependent. HES may decrease platelet adhesion both by reducing plasma levels of von Willebrand factor and by coating platelet membranes. HES may also facilitate thrombolysis. Coagulopathies induced by HES are said to be dose dependent and are usually described with doses exceeding 20 ml/Kg/d(Baldassarre, Vincent, 1997).

San Felippo and Suberviola(San Filippo, Suberviola, 1987) reported a patient with cerebral vasospasm who developed gingival bleeding after 10 days of HES infusion at a rate of 1000 ml/d.

It must however be emphasised that, most bleeding complications with HES have been reported from the United States where only the slowly degradable, high molecular weight form HES 480/0.7 is available for plasma volume expansion. These side effects appear to be avoided by the use of medium or lower molecular weight HES that is easier to degrade (Treib et al., 1999).

Data collected in vivo suggests that, from a rheological perspective, highly substituted medium and high molecular weight HES are less desirable, because they increase plasma viscosity. Medium or low molecular weight starches with a low degree of substitution and a resulting low in vivo molecular weight, have better rheological properties (Treib et al., 1999). It must also be remembered that when selecting a colloid consideration must be given to its half life of elimination, especially if side effects which are potentially life threatening, are likely to occur.

Baldassarre et al (Baldassarre, Vincent, 1997) described a patient treated with 2500ml of HES (450 kD) over 3 days for the management of acute cerebral vasospasm who developed a significant coagulopathy. The patient still had a concentration of HES in the blood 2 days after discontinuing therapy. In this patient, APPT was prolonged, D-Dimers and fibrinogen were normal, and factors VIII, XI and XII significantly reduced.

In the past 2 decades, haemodilution therapy by plasma expanders such as dextrans and HES has become important in the treatment of ischaemic stroke. Dextrans have a wide range of molecular weights. Their clinical efficacy is mainly related to plasma expansion by colloid osmotic activity, to a molecular weight dependent influence on erythrocyte aggregation, and to

inhibition of platelet activity. Dextran are mainly eliminated by the kidneys and this elimination process depends on molecular weight. There is only a minor and slow biotransformation by dextranase in the spleen and liver(Kroemer et al., 1987).

In the treatment of ischaemic stroke with haemodilution long term infusions are required, as the microcirculation of the penumbra is impaired over several days. Kroemer et al(Kroemer et al., 1987) investigated the effects of the long term administration of dextran and HES. With dextran, the plasma viscosity increased and was linearly correlated with the total plasma concentration. With HES the plasma viscosity fell and was not related to the plasma concentration. The haemodiluting effect, as indicated by a decrease of the haematocrit, was 22% and 16.8% for dextran and HES respectively. This data suggested that there were several advantages in using HES compared with dextran in the haemodilution therapy for ischaemic stroke.

Reasons for the described differences might be the different molecular attraction between fibrin molecules and the applied solution. Another explanation might be changes in the polymerisation process of fibrin. HES changes the fibrin fibre mass:length ratios and clot elastic modulus, which changes the tensile properties of the clot(Konrad et al., 2000).

Preoperative normovolaemic acute haemodilution (PNAH) helps to reduce blood transfusion requirements in patients undergoing major surgery. PNAH reduces mean packed cell volume (PCV) leading to a decrease in blood viscosity. This in turn increases blood flow, assuming that the Hagan-Poiseuille equation is observed. The linear decrease in PCV results in an exponential improvement in the rheological properties of blood, particularly in

the PCV range 30-45%, with conservation of oxygen delivery from increased cardiac output despite the dilutional reduction in blood oxygen capacity. As a result of dilution, blood lost during surgery contains plasma substitute, but fewer cells and plasma elements.

Freyburger et al (Freyburger et al., 1996) investigated the rheological properties of commonly used plasma substitutes in vivo. They studied 40 patients undergoing elective aortic reconstruction. The patients were given, 4% human albumin (HA), 3.5% dextran 40 (Dex 40), 6% dextran 60 (Dex 60), 6% hydroxyethylstarch 200 (HES) or modified fluid gelatin (Gel) to achieve PNAH to produce a PCV of 30%. Mean volumes of more than 1000 ml were infused. Blood samples were obtained before infusion, immediately after, and 1.5 hours after the end of haemodilution. The following variables were measured: PCV, plasma viscosity, whole blood viscosity, and erythrocyte aggregation. The 5 substitutes had very different effects on red blood cell aggregation and low shear rate viscosity at corrected PCV. Red blood cell aggregation was reduced in the presence of HA and Dex 40, but was increased moderately to markedly in the presence of the other substitutes in the following order: HES < Dex 60 < Gel. The influence on the rheological conditions on tissue oxygenation was assessed by measuring the concentration of lactic acid. This was unchanged after PNAH with HA or Dex 40, but was increased in the presence of HES, Dex 60 or Gel.

Erythrocyte aggregation tends to occur at very low shear rates encountered in the venous circulation, where most thrombi occur. In whole blood, red cell aggregation results from cross linking of cells via interactions of their membranes with plasma fibrinogen, globulins and macromolecules. An

energetic equilibrium is set up between the aggregation forces (adhesive properties of macromolecules, where plasma substitutes operate) and repulsive forces (electrical charges on the red blood cell surface and shear stress). Erythrocyte aggregation is a complex variable because of its relationship with blood flow. Thus, any variable modifying red cell aggregation may influence tissue perfusion by slowing down circulation and reducing capillary flow. Dex 60 and Gel facilitate red blood cell aggregation in vitro by reducing the electrostatic repulsive forces after adsorption onto cell membranes. HES has a less consistent rheological effect with a decrease in low shear rate viscosity at corrected PCV and no change in aggregation time, in spite of increased cohesion of the aggregates(Freyburger et al., 1996).

Turkan et al(Turkan et al., 1999) studied the effect of a 500ml 6% HES infusion, in 20 patients undergoing elective abdominal surgery. Blood samples were taken immediately before and after the HES infusion approximately 1 hour prior to surgery. The following variables were assessed: platelet count, PT, APPT, fibrinogen, Factor VIII and platelet aggregation. They observed a decrease in fibrinogen concentration, an increase in APPT, and a decrease in factor VIII activity following the infusion. The mean maximum platelet aggregations induced by all aggregating agents were decreased significantly.

Stimulation of fibrinolysis may be caused by enhanced systemic fibrinolytic activity due to an increase in plasminogen activator, a decrease in PAI, or by stimulation of fibrin proteolysis caused by the incorporation of HES into fibrin clots, a mechanism reported for dextran(Carlin, Bang, 1980).

The degree of haemodilution may be crucial to the observed coagulation changes. One might hypothesise, that the anticoagulant activity of blood may be compromised more than the procoagulant activity during moderate haemodilution, resulting in a hypercoagulable state. With advanced haemodilution, procoagulant activity may become diluted and the net result at profound haemodilution is compromised blood coagulation.

The results of this study are in broad agreement with previous reported findings. Interestingly, there was little observed change in R, suggesting that routine coagulation studies such as PT and APPT would have failed to observe the changes in coagulation found with haemodilution using TEG.

The value for K was shortened and Angle increased for Gelfusion and 0.9% normal saline. These TEG variables are concerned with the kinetics of clot development. They are indication of the speed of fibrin build up and cross-linking. To this extent both Gelfusion and 0.9% normal saline haemodilution is promoting coagulation, with the greatest changes seen with Gelfusion.

However, the measures of clot strength MA and G are reduced with both Gelfusion and 0.9% normal saline. Thus, although both promote fibrin formation and cross-linking, it appears that the final structure is not as strong as seen with undiluted citrated whole blood. Final clot strength is closely related to fibrinogen concentration and platelet number and function. As discussed, there is good experimental evidence to explain why this may be so. Certainly there is strong evidence that Gelfusion and other gelatin based colloids react with and bind to fibronectin, thus being incorporated into the final clot structure, and thus perhaps weakening it. There is also evidence of an antiplatelet effect on both adhesion and aggregation.

HES 200/0.5 was found to be profoundly hypocoagulable in all TEG variables except the R time. This may be explained by the fact that HES has been shown to decrease platelet adhesiveness and aggregation, and decrease both factor VIII:C and factor VIII:RC levels.

The clinical implications of these findings are potentially very important as our results suggest that infusion of crystalloid solutions, the common practice in the management of SAH, may increase the risk of ischaemic or thrombotic complications in patients already predisposed to these events. It may be that alternate fluid regimens, or combination regimens should be sort to optimise the management in these patients to prevent secondary insult. In those patients with symptoms of cerebral vasospasm it may prove possible to increase cerebral perfusion and oxygenation by changing patients intravenous fluid protocol to promote a hypocoagulable profile.

In addition, when examining clinical studies relating to coagulation changes, one must be aware of the fluid regimens that have been used and be wary of cases where normal saline has been used for the control population.

Conclusions

Haemodilution does alter coagulation measured in vitro using TEG.

HES induces a profound hypocoagulable change in the haemostatic response as measured by TEG.

Gelfusion and 0.9% normal saline promote the kinetics of clot development and fibrin formation, but ultimately reduce final clot strength.

The coagulation effects observed are not particular to crystalloids or colloids.

As has been demonstrated in this study, the two colloids had strikingly different effects on the coagulation profile following haemodilution.

TEG by providing evidence of the dynamics of clot formation, and examining whole blood and not just plasma, is extremely valuable in studying the effects of intravenous fluids on the haemostatic process.

As coagulation variables are dynamic, especially in the perioperative setting, extrapolation of our data to the in vivo effects is difficult.

Summary of control and experimental studies and directions of future research

I have examined the use of Thromboelastography (TEG[®]) as an in-vitro bench test in monitoring the haemostatic process. TEG[®] is a near-patient test of coagulation. It is easy to perform, relatively inexpensive, and can provide information on a patient's coagulation status within 30 minutes. TEG[®] can provide information on both coagulation and fibrinolysis from a single tracing.

Standard coagulation assays such as the Prothrombin time (PT) and activated partial thromboplastin time (APTT) are plasma tests designed with substitutes for platelet surfaces. Haemostasis is a balance between the coagulation system forming fibrin and the fibrinolytic system breaking it down. Measuring isolated components of these systems may ignore the interactive nature of the process. In addition, quantitatively measuring a specific factor or protein gives no indication of its function in vivo. Functional activity is also dependent on the presence and activity of activators, inhibitors and cellular elements. Clotting is a dynamic process which is difficult to measure using stated end points, which provide no information about the quality of the clot or the dynamics of its formation. TEG[®] provides an integrated profile of the whole haemostatic process. TEG[®] provides additional valuable information which can not be obtained readily from routine haematological indices, and is complimentary to them. TEG[®] appears to be particularly useful in detecting hypercoagulable clinical states.

Experience with TEG[®] has indicated that to get reproducible results all aspects of sample handling must be standardised. This must include sample

collection (tourniquet/no tourniquet, Vacutainer™/glass tubes), sample handling (mixing/agitation/storage temperature), and length of sample storage prior to analysis. Care must be taken to avoid heparin contamination from arterial lines, and with the addition of the incorrect volume of blood to citrated collecting tubes or calcium chloride to the cup. If all aspects of sample handling are standardised then my control studies indicate that TEG® is an extremely reproducible measure of haemostasis. The reproducibility of TEG® is at least as good as the more frequently used laboratory coagulation profiles.

I would encourage all departments using TEG® to generate their own 'normal' reference ranges for their sample collection and handling techniques and ignore the commercial ranges generated by the manufacturers software, which in my experience is extremely sensitive to these forces.

TEG® analysis in the control population studied showed a highly significant sex difference. For the TEG parameters K, Angle, MA, tMA and G female controls showed a hypercoagulable tendency when compared with males. This could not be explained by pregnancy or OCP use. Age appeared to have no significant effect on TEG® analysis. Similar sex differences in coagulation were also observed in the SAH patients studied. The routinely performed coagulation tests such as PT, APTT, INR and fibrinogen, do not have reference ranges for men and women, and we aim to investigate these TEG® differences further.

The experimental studies demonstrate that measured by TEG®, a consecutive series of SAH patients became increasingly hypercoagulable over time (maximum 21 days) following the ictus, despite haematological

evidence of haemodilution. This prothrombotic tendency had reversed by 3 months. Both PT and APTT were insensitive to this hypercoagulable state.

Plasma assays of coagulation and fibrinolysis show that both of these processes are stimulated following a SAH. However, coagulation is proportionally stimulated to a greater extent to tip the balance towards this process for the first 21 days following a SAH.

The treatment aim of haemodilution and hypervolaemia was comprehensively achieved with the fluid regimen employed. The haematocrit fell from a mean value of 0.39 l/l in the first week, to 0.32 l/l in the third week (0.42 l/l at 3 months). This is paralleled by falls in haemoglobin concentration and red cell count. It is interesting to note that despite powerful evidence of haemodilution during the first 21 days following the SAH, both the fibrinogen concentration and the platelet count rise abruptly. The functional result of this is a prothrombotic response.

I have investigated the effect of operative intervention on the trend of increased coagulability following SAH. It might be expected that the stress of surgery, pain, anaesthesia and the release of thromboplastins from the brain substance would produce a further increase in coagulation profiles. Any further increase in coagulation would predispose to thrombosis, and potential ischaemic outcome. In the operative study, I observed little change in TEG parameters around the time of surgery. These findings are consistent with those of other authors investigating the changes at similar time periods around open abdominal and pelvic surgery in the absence of large volume blood loss. I observed no statistically significant difference in the postoperative trend in TEG variables between the surgically and non-

surgically treated patients. This suggests that surgery was not additive to the profile of increased coagulation observed in SAH patients. However, the trend was for the surgical patients to be more hypercoagulable. This may be explained by the fact that the surgically treated group had a more hypercoagulable initial mean when their first TEG sample was performed.

Thus, I have failed to identify a difference between the surgical and non-surgically treated groups. However, an alternative explanation for this may be in the mixture of the non-operative patients. This group comprises both the best (angiogram negative) and worst (those treated conservatively because of their poor condition and prognosis) patients in a SAH population. It may be that if a pure perimesencephalic haemorrhage population, or a grade-matched embolisation population, were compared with the surgical group, then a significant difference might have been found. Due to a limitation in numbers, these comparisons have not been possible in this study. It has been suggested that the outcome following embolisation is better than after intracranial surgery for cerebral aneurysms, and that the risks of ischaemia are less. It is only speculative at this time how the influence of these treatment modalities on coagulation might influence patient outcome.

It has been suggested that there is an association between makers of haemostatic activation following SAH and clinical outcome. In this study, I have tried to bring these theories together and correlate the blood load (measured by Fisher grade), with haemostatic activation (measured using TEG). I failed to show any statistically significant relationship however, the means for each TEG variable did become increasingly hypercoagulable from

Fisher grades 1 through to 3. Fisher grade 4 patients did not follow this trend, and had a mean value roughly between those in grade 1 and grade 2 for each TEG variable. We continue to recruit SAH patients to investigate this relationship in more detail and to ensure a statistically significant relationship has not been missed because each Fisher grade group was not equally represented in the initial sample.

The final experimental study investigated in vitro whether haemodilution 'per se' has an intrinsic effect on coagulation specific to the diluent itself and thus may be contributing to the observed changes in coagulation following SAH. At a 30% haemodilution HES induced a profound hypocoagulable change in the haemostatic response as measured by TEG. Gelfusion and 0.9% normal saline promoted the kinetics of clot development and fibrin formation, but ultimately reduced final clot strength. TEG by providing evidence of the dynamics of clot formation, and examining whole blood and not just plasma, is extremely valuable in studying the effects of intravenous fluids on the haemostatic process. These results have now been repeated in vitro on SAH patients. We now aim to investigate if the coagulation profiles of SAH patients can be manipulated in vivo with differing fluid replacement regimens. To further investigate the gender differences in coagulation we propose to study TEG[®] parameters in pre and postmenopausal women not taking HRT and an age matched (postmenopausal) male cohort. In addition we have an ongoing project examining TEG[®] profiles in different stages of the menstrual cycle in subjects taking and not taking the oral contraceptive pill.

We continue to recruit SAH patients following the same protocol employed in the experimental studies. We will compare coagulation profiles in a cohort of

grade-matched perimesencephalic (angiogram negative) haemorrhage and angiogram positive patients. In addition we will compare the post-procedure coagulation profiles in grade-matched operative and embolisation cohorts.

We continue to recruit SAH patients to further investigate the relationship between Fisher grade and haematological activation to ensure a significant correlation was not missed due to each grade not being equally represented in the initial sample population.

To further investigate the effect of intravenous fluid therapy on coagulation in SAH patients we propose the following study.

The in vivo effects of haemodilution on haemostasis following subarachnoid haemorrhage, as measured by thromboelastography.

Introduction

The cornerstone of management in patients presenting with a SAH is the prevention of delayed ischaemic neurological deficit (DIND). This is achieved by so-called “triple – H” therapy i.e. Hypertension, Haemodilution and Hypervolaemia. In clinical practice this means large volumes of IV fluid replacement. Different clinicians employ different fluid regimes, some preferring crystalloids, some colloids and some combinations.

The effect of IV fluids on coagulation have not been widely investigated, mainly due to the fact that the routine employed coagulation studies such as PT and APTT are quite insensitive. If a particular colloid induced a hypercoagulable response following its administration one might assume that this could predispose to ischaemic complications in a vasospastic population such as patients following SAH. The aim of this study is to investigate the

effects of the various fluids used in normal clinical practice on whole blood coagulation as measured by TEG.

Methods

Participants

30 patients admitted to the National Hospital for Neurology and Neurosurgery with a diagnosis of SAH will be recruited into the study.

Procedure

It is normal practice for SAH patients to be managed with a minimum of 3 litres of normal saline in a 24 hour period. Thus, this will be taken as the control population. A pre-fluid administration blood sample will be taken from each of these patients and a control TEG analysis performed. Each patient will then be administered a unit of one of the following routinely used colloid solutions (Dextran 40, Hexastarch or Gelofusine) over a one-hour period. On completion of this, the patient will be left for a further hour and then a second TEG analysis will be performed. Analysis of TEG data will then take place between the control sample and the post-fluid sample, to assess for the in vivo effect of each colloid on coagulation. Following a “wash-out” period of three days, the procedure will be repeated with the same colloid until the patient no longer requires intravenous fluids. In addition the trend in TEG indices in control samples over the first 21 days following the ictus will be calculated and compared between the 3 groups.

Statistical Analysis

All data will be transferred directly to and analysed using Microsoft Excel. A paired t-test will be used to make a comparison between the mean difference between TEG parameters for the pre- and post- fluid administration. The next

analysis involved calculating the rate of change of each of the TEG variables over time for the first 21 days following the SAH. This will be calculated by regressing each clotting parameter in turn on the time since SAH, for each individual separately. A single sample t-test was used to see if the mean rates of change differed significantly from zero for the control samples. A significance level of $p < 0.05$ was taken.

To further investigate the effect of intracranial surgery and coil embolisation on the coagulation profiles of SAH patients we propose the following study.

Comparison of the procoagulant effects of embolisation and surgery in patients with SAH.

Aneurysm rebleeding and delayed ischaemic neurological deficit (DIND) attributable to clinical vasospasm are major causes of morbidity among patients who survive the initial effects of aneurysm rupture. The optimal timing of surgery for acutely ruptured aneurysms is still the subject of debate, because of the effects of vasospasm, which most commonly occurs 3 to 10 days following the ictus and has a detrimental effect on patient outcomes. The International Co-operative Study on the Timing of Aneurysm Surgery suggested that results depended on the time interval between treatment and subarachnoid haemorrhage (SAH)(Kassell et al., 1990a). Early surgery reduced the risk of rebleeding, but the technical results of surgery were better if surgery was delayed until at least 10 days following the SAH. However, overall the advantages of delayed surgery were negated by rebleeding during the preoperative period.

Background to present research.

We hypothesise that following SAH there is an undefined period of increasing hypercoagulability, which if present would predispose to ischaemic stroke.

Methods. We have carried out a prospective, observational study in 67 consecutive patients with a primary diagnosis of SAH. Blood was taken at 4 time periods (<48hours, 4-5, 9-10 and 15-16 days) following the ictus, and on regular intervals during the hospital stay. In addition blood was sampled 3 months from the ictus. At each time point a Thromboelastograph (TEG) profile, INR and APTT assay was performed.

Of these 67 patients, the cause of the SAH was a ruptured cerebral aneurysm in 56, an AVM in 2, the angiogram was negative in 6, and the cause was undermined in 3 patients.

Findings. The routine clotting studies INR and APTT showed no change over time. TEG analysis demonstrated the development of a sequentially increasing hypercoagulable state in the first 21 days following SAH. There were statistically significant changes in the following TEG variables K time ($p=0.001$), Angle ($p<0.0001$), MA ($p<0.0001$), tMA ($p=0.0001$) and G ($p<0.0001$). Following the ictus the patients were significantly hypercoagulable in TEG indices than when sampled at 3 months ($p<0.001$).

Interpretation. There are sound methodological reasons to explain why routine coagulation studies may not be as sensitive as TEG analysis in identifying a hypercoagulable state. A state of increased thrombogenicity may increase the risk of cerebral ischaemia and infarction by increasing the incidence of micro-thrombotic events.

Although the risk of morbidity following surgery in the time of maximal vasospasm is increased, recent studies have suggested that there is no increased procedural risk with coil embolisation during this time (Baltsavias et al., 2000; Wikholm et al., 2000). Why should this be? Is it that surgery further increases the developing hypercoagulable state, leading to ischaemia and stroke. Perhaps manipulation of the brain itself, causing release of thromboplastins during surgery is responsible for this. An alternative explanation maybe the reduced fluid requirements of patients undergoing embolisation compared with surgical patients, or the increased stress response induced by surgery. Intravenous fluids are well known to effect coagulation. Or perhaps the use of heparin in coiled patients (something that is impossible in surgical candidates), negates the developing hypercoagulable state which we have shown in SAH patients. Thus, reducing ischaemic complications and improving patient outcome.

We propose a study using TEG to compare coagulation activation in SAH patients undergoing embolisation and surgery in grade and sex matched populations.

We will study 30 surgical and 30 coiled aneurysmal SAH patients. Blood will be sampled at <48hours, 4-5, 9-10, 15-16 days and 3 months following the ictus. In addition blood will be sampled on induction of the surgical/coiling procedure, 120 minutes later and 24 hours later. Blood will be profiled for FBC, biochemical indices, PT, APTT, Fibrinogen, TEG, TAT, P1+2, D-Dimer, and PAP. Statistical analysis will be performed to examine the coagulation changes around these procedures. In addition the serial coagulation profiles of these two groups will be compared. We aim to investigate whether

embolisation provides protection from ischaemia by reducing activation of coagulation systems.

Appendix I

WFNS grading of SAH (Lal, 1980)

Group 1. No blood detected.

WFNS grade	GCS	Motor deficit
1	15	absent
2	14--13	absent
3	14--13	present
4	12--7	present or absent
5	6--3	present or absent

(Drake, 1988)

Appendix II

Fisher Grading(Fisher et al., 1980).

Group 1. No blood detected.

Group 2. A diffuse disposition or thin layer with all vertical layers of blood (interhemispheric fissure, insular cistern, ambient cistern) less than 1mm thick.

Group 3. Localised clots and/or vertical layers of blood 1mm or greater in thickness.

Group 4. Diffuse or no subarachnoid blood, but with intracerebral or intraventricular clots.

Appendix III

Normal TEG ranges

R (Secs)	450-690
K (Secs)	120-450
Angle (deg)	36-48
MA (mm)	34-46
G (dyn/cm²)	2575-4259

The aim of the study is to determine which factors cause the blood to clot more quickly after this type of brain injury. Increased blood coagulation leads to further stroke and may be reversed with drugs leading to reduced disability and increased survival in these patients.

Patients entering the study will have a small additional amount (5ml) of blood taken on each routine occasion when blood would normally be taken during their hospital stay. The only occasions when a blood sample will be taken in addition to the present routine samples are at 50 days and 90 days following the injury. This volume of blood is replaced within hours of sampling and will not alter the subject's chances of making a good recovery from their illness.

Your participation in the trial is entirely voluntary. You are free to decline to enter or to withdraw from the study at any time without having to give a reason. If you choose not to enter the trial, or to withdraw once entered, this will in no way affect your future medical care. All information regarding your medical records will be treated as strictly confidential and will only be used for medical purposes. Your medical records may be inspected by competent authorities and properly authorised persons, but if any information is released this will be done so in coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your legal rights.

The two investigators in this study, Mr Andrew McEvoy and Mr N D Kitchen can be contacted during working hours via Mr Kitchen's secretary at the National Hospital on 0171 837 3611 ext3021. In cases of emergency, Mr McEvoy can be contacted out of hours via the National Hospital switchboard and internal pager 24 hours per day.

Appendix IV

CONSENT BY VOLUNTEER.

INVESTIGATORS: Mr Andrew McEvoy FRCS
Mr Neil Kitchen MD FRCS (SN)

We would like to invite you to take part in a study to determine the factors which alter the ability of the blood to clot during brain injury.

Traumatic brain injury and brain haemorrhage (stroke) accounts for a substantial and often devastating disease burden in young and middle aged adults. Because little can be done about the initial injury, treatment must be directed towards preventing secondary events which themselves lead to further disability.

The aim of the study is to examine which factors cause the blood to clot more quickly after this type of brain injury. Increased blood coagulation leads to further stroke and may be reversed with drugs leading to reduced disability and increased survival in these patients.

Patients entering the study will have a small additional amount (5mls) of blood taken on each routine occasion when blood would normally be taken during their hospital stay. The only occasion when a blood sample will be taken in addition to the present routine samples are at 60 days and 90 days following the injury. This volume of blood is replaced within hours of sampling and will not alter the subject's chances of making a good recovery from their illness.

Your participation in the trial is entirely voluntary. You are free to decline to enter or to withdraw from the study at any time without having to give a reason. If you choose not to enter the trial, or to withdraw once entered, this will in no way affect your future medical care. All information regarding your medical records will be treated as strictly confidential and will only be used for Medical purposes. Your medical records may be inspected by competent authorities and properly authorised persons, but if any information is released this will be done so in coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your legal rights.

The two investigators in this study, Mr Andrew McEvoy and Mr N D Kitchen can be contacted during working hours via Mr Kitchen's secretary at the National Hospital on ext . In cases of emergency, Mr McEvoy can be contacted out of hours via the National Hospital switchboard and aircall pager 24 hours per day.

CONSENT BY RELATIVE.

INVESTIGATORS: Mr Andrew McEvoy FRCS
Mr Neil Kitchen MD FRCS (SN)

We would like to invite you to take part in a study to determine the factors which alter the ability of the blood to clot during brain injury.

Traumatic brain injury and brain haemorrhage (stroke) accounts for a substantial and often devastating disease burden in young and middle aged adults. Because little can be done about the initial injury, treatment must be directed towards preventing secondary events which themselves lead to further disability.

The aim of the study is to examine which factors cause the blood to clot more quickly after this type of brain injury. Increased blood coagulation leads to further stroke and may be reversed with drugs leading to reduced disability and increased survival in these patients.

Patients entering the study will have a small additional amount (5mls) of blood taken on each routine occasion when blood would normally be taken during their hospital stay. The only occasion when a blood sample will be taken in addition to the present routine samples are at 60 days and 90 days following the injury. This volume of blood is replaced within hours of sampling and will not alter the subject's chances of making a good recovery from their illness.

You are free to agree to your relative being entered or to withdraw them from the study at any time without having to give a reason. If you choose not to enter your relative into the trial, or to withdraw them once entered, this will in no way affect their future medical care. Your relative will be informed of his/her involvement in the study, and your agreement, should they regain consciousness. All information regarding their medical records will be treated as strictly confidential and will only be used for medical purposes. Their medical records may be inspected by competent authorities and properly authorised persons, but if any information is released this will be done so in coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your relative's legal rights.

The two investigators in this study, Mr Andrew McEvoy and Mr N D Kitchen can be contacted during working hours via Mr Kitchen's secretary at the National Hospital on ext. . In cases of emergency, Mr McEvoy can be contacted out of hours via the National Hospital switchboard and air call pager 24 hours per day.

Appendix V

Glasgow outcome score

Grade	Neurological status
1	Good recovery; patient can lead a full and independent life with or without minimal neurological deficit
2	Moderately disabled; patient has neurological or intellectual impairment but is independent
3	Severely disabled patient, conscious but totally dependent on others to get through daily activities
4	Vegetative survival
5	Dead

(Jennett, Bond, 1975)

Appendix VI

Regression Statistics	
Multiple R	0.27
R Square	0.07
Adjusted R Square	-0.04
Standard Error	43209.63
Observations	20

ANOVA

	df	SS	MS	F	Significance F
Regression	2	2.41E+09	1.21E+09	0.65	0.54
Residual	17	3.17E+10	1.87E+09		
Total	19	3.42E+10			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-25745.27	50604.19	-0.51	0.61	-132511	81020.4
sex	12420.25	21462.71	0.58	0.57	-32862.2	57702.7
age	733.96	1167.49	0.63	0.54	-1729.23	3197.14

Table 50. Analysis of the effects of gender and age on the mean difference between R for day 0 and day 14.

Using $p < 0.05$ to determine significance, regression analysis of R for effects of gender and age revealed that neither gender nor age produced a significant effect on R ($p > 0.05$).

Regression Statistics	
Multiple R	0.22
R Square	0.05
Adjusted R Square	-0.06
Standard Error	4616.56
Observations	20

ANOVA

	df	SS	MS	F	Significance F
Regression	2	20004672	10002336	0.47	0.63
Residual	17	362314408	21312612		
Total	19	382319080			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-1277.13	5406.60	-0.24	0.82	-12684.08	10130
sex	2212.87	2293.10	0.97	0.35	-2625.15	7050.9
age	-42.73	124.74	-0.34	0.74	-305.91	220.45

Table 51. Analysis of the effects of gender and age on the mean difference between K for day 0 and day 14.

Using $p < 0.05$ to determine significance, regression analysis of K for effects of gender and age revealed that neither gender nor age produced a significant effect on K ($p > 0.05$).

Regression Statistics	
Multiple R	0.18
R Square	0.03
Adjusted R Square	-0.08
Standard Error	77.04
Observations	20

ANOVA

	df	SS	MS	F	Significance F
Regression	2	3294.09	1647.04	0.28	0.76
Residual	17	100906.09	5935.65		
Total	19	104200.18			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	9.27	90.22	0.10	0.92	-181.09	199.64
sex	28.51	38.25	0.74	0.47	-52.23	109.24
age	-0.65	2.08	-0.31	0.76	-5.05	3.74

Table 52. Analysis of the effects of gender and age on the mean difference between Angle for day 0 and day 14.

Using $p < 0.05$ to determine significance, regression analysis of Angle for effects of gender and age revealed that neither gender nor age produced a significant effect on Angle ($p > 0.05$).

Regression Statistics	
Multiple R	0.36
R Square	0.13
Adjusted R Square	0.03
Standard Error	109.86
Observations	20

ANOVA

	df	SS	MS	F	Significance F
Regression	2	31427.31	15713.65	1.30	0.30
Residual	17	205187.5	12069.85		
Total	19	236614.81			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-115.83	128.66	-0.90	0.38	-387.29	155.63
sex	86.83	54.57	1.59	0.13	-28.30	201.96
age	-1.34	2.97	-0.45	0.66	-7.60	4.92

Table 53. Analysis of the effects of gender and age on the mean difference between MA for day 0 and day 14.

Using $p < 0.05$ to determine significance, regression analysis of MA for effects of gender and age revealed that neither gender nor age produced a significant effect on MA ($p > 0.05$).

Regression Statistics	
Multiple R	0.27
R Square	0.07
Adjusted R Square	-0.03
Standard Error	8165988
Observations	20

ANOVA

	df	SS	MS	F	Significance F
Regression	2	9.10E+13	4.55E+13	0.68	0.52
Residual	17	1.13E+15	6.67E+13		
Total	19	1.23E+15			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-4349734	9563451	-0.45	0.65	-24526881	15827413
sex	4736649	4056139	1.17	0.26	-3821068	13294366
age	-109631.9	220638	-0.50	0.63	-575138	355874.12

Table 54. Analysis of the effects of gender and age on the mean difference between G for day 0 and day 14.

Using $p < 0.05$ to determine significance, regression analysis of G for effects of gender and age revealed that neither gender nor age produced a significant effect on G ($p > 0.05$).

Appendix VII

t-Test: Paired Two Sample for Means

	<i>R</i>	<i>R NS</i>
Mean	658.9	667.95
Variance	15021.67	18136.05
Observations	20	20
Pearson Correlation	0.345433	
Hypothesized Mean Difference	0	
df	19	
t Stat	-0.2744	
P(T<=t) one-tail	0.393368	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.786737	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	<i>R</i>	<i>R Gel</i>
Mean	658.9	607.85
Variance	15021.67	7194.766
Observations	20	20
Pearson Correlation	0.56484	
Hypothesized Mean Difference	0	
df	19	
t Stat	2.230954	
P(T<=t) one-tail	0.018966	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.037932	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	<i>R</i>	<i>R HES</i>
Mean	658.9	679.75
Variance	15021.67	15456.83
Observations	20	20
Pearson Correlation	0.466701	
Hypothesized Mean Difference	0	
df	19	
t Stat	-0.73134	
P(T<=t) one-tail	0.236748	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.473495	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	<i>R NS</i>	<i>R Gel</i>
Mean	667.95	607.85
Variance	18136.05	7194.766
Observations	20	20
Pearson Correlation	0.449762	
Hypothesized Mean Difference	0	
df	19	
t Stat	2.190488	
P(T<=t) one-tail	0.020581	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.041162	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	<i>R NS</i>	<i>R HES</i>
Mean	667.95	679.75
Variance	18136.05	15456.83
Observations	20	20
Pearson Correlation	0.63865	
Hypothesized Mean Difference	0	
df	19	
t Stat	-0.47763	
P(T<=t) one-tail	0.319182	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.638363	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	<i>R Gel</i>	<i>R HES</i>
Mean	607.85	679.75
Variance	7194.766	15456.83
Observations	20	20
Pearson Correlation	0.541078	
Hypothesized Mean Difference	0	
df	19	
t Stat	-3.03296	
P(T<=t) one-tail	0.003421	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.006843	
t Critical two-tail	2.093025	

Table 55. t-tests for the TEG variable R for undiluted and diluted samples.

NS = Normal saline, Gel = Gelfusion, HES = Hydroxyethylstarch.

t-Test: Paired Two Sample for Means

	Ang	Ang NS
Mean	47.175	48.625
Variance	62.58618	38.18092
Observations	20	20
Pearson Correlation	0.7209	
Hypothesized Mean Difference	0	
df	19	
t Stat	-1.1783	
P(T<=t) one-tail	0.126612	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.253224	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	Ang	Ang Gel
Mean	47.175	54.15
Variance	62.58618	44.60789
Observations	20	20
Pearson Correlation	0.60087	
Hypothesized Mean Difference	0	
df	19	
t Stat	-4.71884	
P(T<=t) one-tail	7.47E-05	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.000149	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	Ang	Ang HES
Mean	47.175	44.825
Variance	62.58618	36.55987
Observations	20	20
Pearson Correlation	0.652592	
Hypothesized Mean Difference	0	
df	19	
t Stat	1.734491	
P(T<=t) one-tail	0.049513	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.099026	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	Ang NS	Ang Gel
Mean	48.625	54.15
Variance	38.18092	44.60789
Observations	20	20
Pearson Correlation	0.568312	
Hypothesized Mean Difference	0	
df	19	
t Stat	-4.12492	
P(T<=t) one-tail	0.000288	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.000576	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	Ang NS	Ang HES
Mean	48.625	44.825
Variance	38.18092	36.55987
Observations	20	20
Pearson Correlation	0.712014	
Hypothesized Mean Difference	0	
df	19	
t Stat	3.661909	
P(T<=t) one-tail	0.000829	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.001658	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	Ang Gel	Ang HES
Mean	54.15	44.825
Variance	44.60789	36.55987
Observations	20	20
Pearson Correlation	0.454552	
Hypothesized Mean Difference	0	
df	19	
t Stat	6.254685	
P(T<=t) one-tail	2.63E-06	
t Critical one-tail	1.729131	
P(T<=t) two-tail	5.25E-06	
t Critical two-tail	2.093025	

Table 57. t-tests for the TEG variable Angle (Ang) for undiluted and diluted samples.

NS = Normal saline, Gel = Gelfusion, HES = Hydroxyethylstarch.

t-Test: Paired Two Sample for Means

	MA	MA NS
Mean	57.025	51.725
Variance	25.22303	12.06513
Observations	20	20
Pearson Correlation	0.602315	
Hypothesized Mean Difference	0	
df	19	
t Stat	5.875542	
P(T<=t) one-tail	5.85E-06	
t Critical one-tail	1.729131	
P(T<=t) two-tail	1.17E-05	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	MA	MA HES
Mean	57.025	47.8
Variance	25.22303	15.27368
Observations	20	20
Pearson Correlation	0.745722	
Hypothesized Mean Difference	0	
df	19	
t Stat	12.31477	
P(T<=t) one-tail	8.36E-11	
t Critical one-tail	1.729131	
P(T<=t) two-tail	1.67E-10	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	MA NS	MA HES
Mean	51.725	47.8
Variance	12.06513	15.27368
Observations	20	20
Pearson Correlation	0.496852	
Hypothesized Mean Difference	0	
df	19	
t Stat	4.71672	
P(T<=t) one-tail	7.51E-05	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.00015	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	MA	MA Gel
Mean	57.025	54.15
Variance	25.22303	20.81842
Observations	20	20
Pearson Correlation	0.604461	
Hypothesized Mean Difference	0	
df	19	
t Stat	3.002391	
P(T<=t) one-tail	0.003661	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.007323	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	MA NS	MA Gel
Mean	51.725	54.15
Variance	12.06513	20.81842
Observations	20	20
Pearson Correlation	0.348114	
Hypothesized Mean Difference	0	
df	19	
t Stat	-2.3201	
P(T<=t) one-tail	0.01581	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.031621	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	MA Gel	MA HES
Mean	54.15	47.8
Variance	20.81842	15.27368
Observations	20	20
Pearson Correlation	0.740397	
Hypothesized Mean Difference	0	
df	19	
t Stat	9.124259	
P(T<=t) one-tail	1.13E-08	
t Critical one-tail	1.729131	
P(T<=t) two-tail	2.26E-08	
t Critical two-tail	2.093025	

Table 58. t-tests for the TEG variable MA for undiluted and diluted samples.

NS = Normal saline, Gel = Gelfusion, HES = Hydroxyethylstarch.

t-Test: Paired Two Sample for Means

	G	G NS
Mean	6643.75	5658.725
Variance	2495220	969424.2
Observations	20	20
Pearson Correlation	0.408187	
Hypothesized Mean Difference	0	
df	19	
t Stat	2.973379	
P(T<=t) one-tail	0.003904	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.007808	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	G	G Gel
Mean	6643.75	5904.2
Variance	2495220	1309572
Observations	20	20
Pearson Correlation	0.721011	
Hypothesized Mean Difference	0	
df	19	
t Stat	3.021602	
P(T<=t) one-tail	0.003509	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.007018	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	G	G HES
Mean	6643.75	4649.15
Variance	2495220	540518.5
Observations	20	20
Pearson Correlation	0.676666	
Hypothesized Mean Difference	0	
df	19	
t Stat	7.372104	
P(T<=t) one-tail	2.76E-07	
t Critical one-tail	1.729131	
P(T<=t) two-tail	5.52E-07	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	G NS	G Gel
Mean	5658.725	5904.2
Variance	969424.2	1309572
Observations	20	20
Pearson Correlation	0.144568	
Hypothesized Mean Difference	0	
df	19	
t Stat	-0.7855	
P(T<=t) one-tail	0.220923	
t Critical one-tail	1.729131	
P(T<=t) two-tail	0.441845	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

	G NS	G HES
Mean	5658.725	4649.15
Variance	969424.2	540518.5
Observations	20	20
Pearson Correlation	0.576528	
Hypothesized Mean Difference	0	
df	19	
t Stat	5.494302	
P(T<=t) one-tail	1.33E-05	
t Critical one-tail	1.729131	
P(T<=t) two-tail	2.67E-05	
t Critical two-tail	2.093025	

t-Test: Paired Two Sample for Means

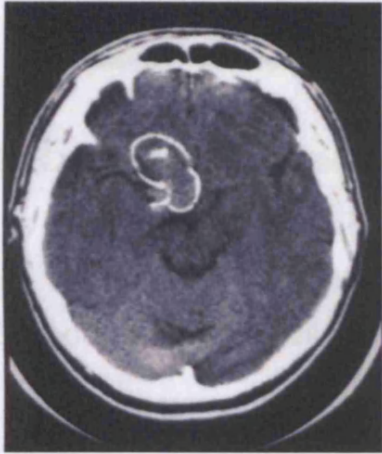
	G Gel	G HES
Mean	5904.2	4649.15
Variance	1309572	540518.5
Observations	20	20
Pearson Correlation	0.681467	
Hypothesized Mean Difference	0	
df	19	
t Stat	6.692273	
P(T<=t) one-tail	1.07E-06	
t Critical one-tail	1.729131	
P(T<=t) two-tail	2.13E-06	
t Critical two-tail	2.093025	

Table 59. t-tests for the TEG variable G for undiluted and diluted samples.

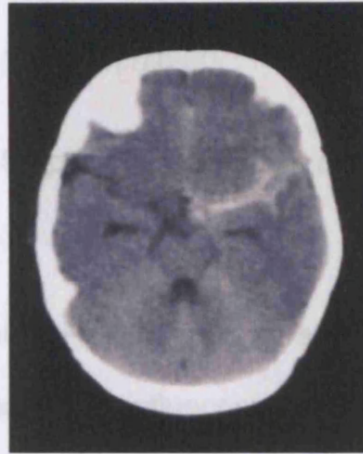
NS = Normal saline, Gel = Gelfusion, HES = Hydroxyethylstarch.

Appendix VIII

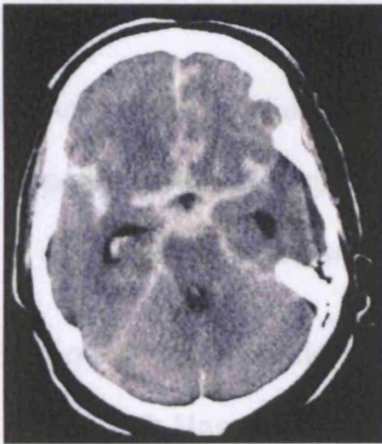
Fisher grades on CT imaging



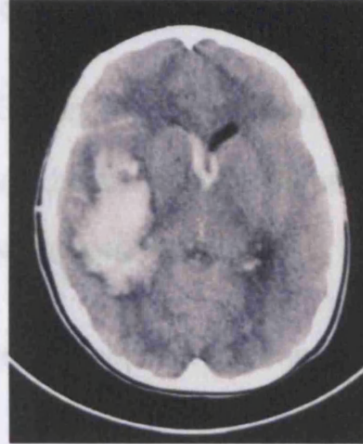
Grade 1



Grade 2.



Grade 3



Grade 4.

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